GENETIC ASPECTS OF PLANT MINERAL NUTRITION

Developments in Plant and Soil Sciences

VOLUME 42

The titles published in this series are listed at the end of this volume.

Genetic Aspects of Plant Mineral Nutrition

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Contributions indicated with an asterisk in the table of contents were first published in *Plant and Soil*, Volume 123 (1990)

Kluwer Academic Publishers
DORDRECHT / BOSTON / LONDON

Library of Congress Cataloging in Publication Data

International Symposium on Genetic Aspects of Plant Mineral Nutrition (3rd : 1988 : Braunschweig, Germany)
Genetic aspects of plant mineral nutrition : proceedings of Third International Symposium on Genetic Aspects of Plant Mineral Nutrition / organized by Institute of Crop Science and Plant Breeding, Federal Research Centre for Agriculture, D-3300 Braunschweig, FRG. June 19-24, 1988 ; editors, N. El Bassam, M. Dambroth and B.C. Loughman.
p. cm. -- (Developments in plant and soil sciences ; 42)
ISBN 0-7923-0785-2 (acid-free paper)
1. Plants--Nutrition--Genetic aspects--Congresses. 2. Plants, Effect of minerals on--Congresses. I. El Bassam, Nasir. II. Dambroth, Manfred. III. Loughman, B. C. IV. Institut für Pflanzenbau und Pflanzenbau und Pflanzenzüchtung (Braunschweig, Germany) V. Title. VI. Series: Developments in plant and soil sciences ; v. 42.
QK867.I424 1988
581.1'335--dc20 90-4836

ISBN-13: 978-94-010-7421-6 e-ISBN-13: 978-94-009-2053-8
DOI: 10.1007/978-94-009-2053-8

Published by Kluwer Academic Publishers,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Kluwer Academic Publishers incorporates
the publishing programmes of Martinus Nijhoff,
Dr W. Junk, D. Reidel, and MTP Press.

Sold and distributed in the U.S.A. and Canada
by Kluwer Academic Publishers,
101 Philip Drive, Norwell, MA 02061, U.S.A.

In all other countries, sold and distributed
by Kluwer Academic Publishers Group,
P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

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Preface

The genetic work that has been carried out with crop plants indicates that many nutritional characteristics are independently inherited and could be selected for a breeding program.

This volume presents the proceedings of the 'Third International Symposium on Genetic Aspects of Plant Mineral Nutrition' held in Braunschweig 1988, and demonstrates the wide acceptance that cultivars respond differently to nutritional and stress factors. An improved response of cultivars to nutrients and stress conditions is accessible via screening, selection and breeding of the available plant genetic resources and will help to reduce the inputs and to protect the environment.

The contribution of the plant breeders in this volume, and especially in Section V 'Germplasm resources and creation of genotypes for specific environmental including low input systems', is evident. This can be considered as genuine progress in the direction of an effective use of nutrients by plants.

We look forward to more advances in our understanding of the mechanisms involved in the nutrient efficiency of crop plants and in the development and improvement of screening techniques at the Fourth Symposium to be held in Australia in 1991.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 1–7.
PLSO PN 122
© 1990 *Kluwer Academic Publishers.*

Genotypic variation in plant productivity and consequences for breeding of 'low-input cultivars'

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Key words: low-input cultivars, nutrient efficiency, landraces, new cultivars, nitrogen utilization efficiency, plant productivity, plant breeding

Abstract

Important characteristics of low input genotypes are a high efficiency of nutrient and water use, of assimilation, a proper assimilate transport and distribution, less losses of assimilates through respiration and adequate nutrient acquisition by the root system. The response of cultivars to inputs is under genetic control and therefore improved response is accessible via screening, selection and breeding.

The current modern cultivars showed a higher nutrient efficiency rate than the old cultivars or landraces and possess also higher yield potential under low levels of inputs. A wide range in nutrient efficiency exists within the recent cultivars and it seems to be possible that a special breeding programme of crop cultivars for low nutrient conditions will be successful.

Factors affecting productivity of plants

Approximately 350,000 plant species have been identified by botanists and about 3,000 of them were used by men as food sources. Currently, 300 species were domesticated as crops in worldwide agriculture and at present about 50 crop species are of major importance. Cereals as a group supply about 65% of dry matter and over 50% of the protein consumed in the world.

The Figures 1a–1d illustrate the worldwide average yields per hectare of 54 crop species (FAO, 1987) and demonstrate the big variation and yields of these crop species. The productivity and yielding capacity of various plants are mainly genetically fixed and controlled by the ecological constraints and the level of the inputs. Comparing the data given in the figures, it is obvious that the worldwide averages of dry matter yields of the most cultivated crops range between 0.26 tons ha^{-1} for hops and 15.6 tons ha^{-1} for sugar cane. Many of these 54 crop species which contain less than 10% to more than 95% moisture achieved higher yields in the last five years. These figures also indicate the necessity for genetic improvement of the productivity of several of the crop species, *e.g.* hops, sesame and flax, if higher yields are to be achieved.

The growth and yield quantum of the crops is determined by their genetic potential. The achievement of this potential is closely related to the environmental conditions and external inputs. Figure 2 summarizes the genetic and ecological components responsible for biomass production. The degree of plant dependence on climatic and environmental factors depends on the genetic structure of both the population and individual genotypes. On the other hand, the environment influences the genotype successfully in different sites (genotype × environment-interaction). Maximum yield achieved in field experiments does not represent a physiological limitation of the present cultivars but demonstrates that portion of the genetic potential which is realizable by the optimal utilization of the present means of cultivation (Dambroth and El Bassam, 1983).

Genetic improvement of plant productivity

Crop productivity has been increased by a combination of improved varieties and technological

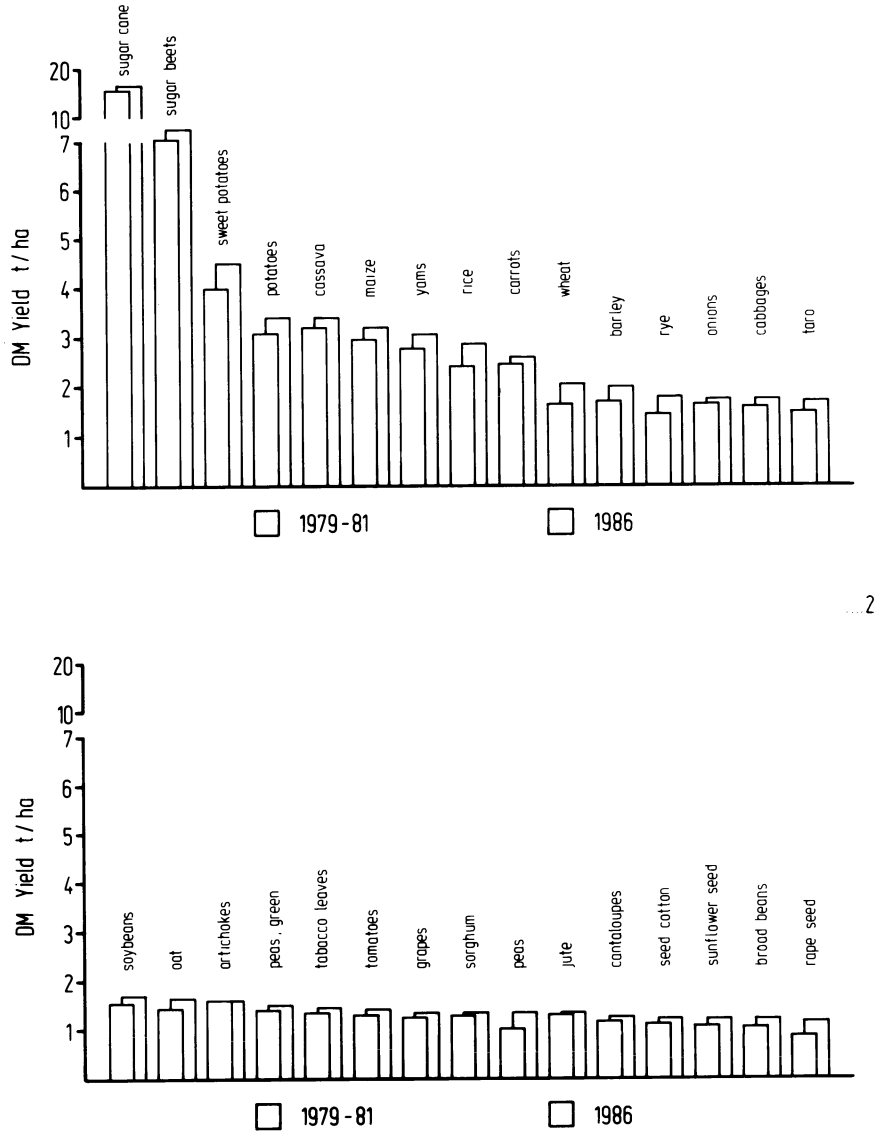
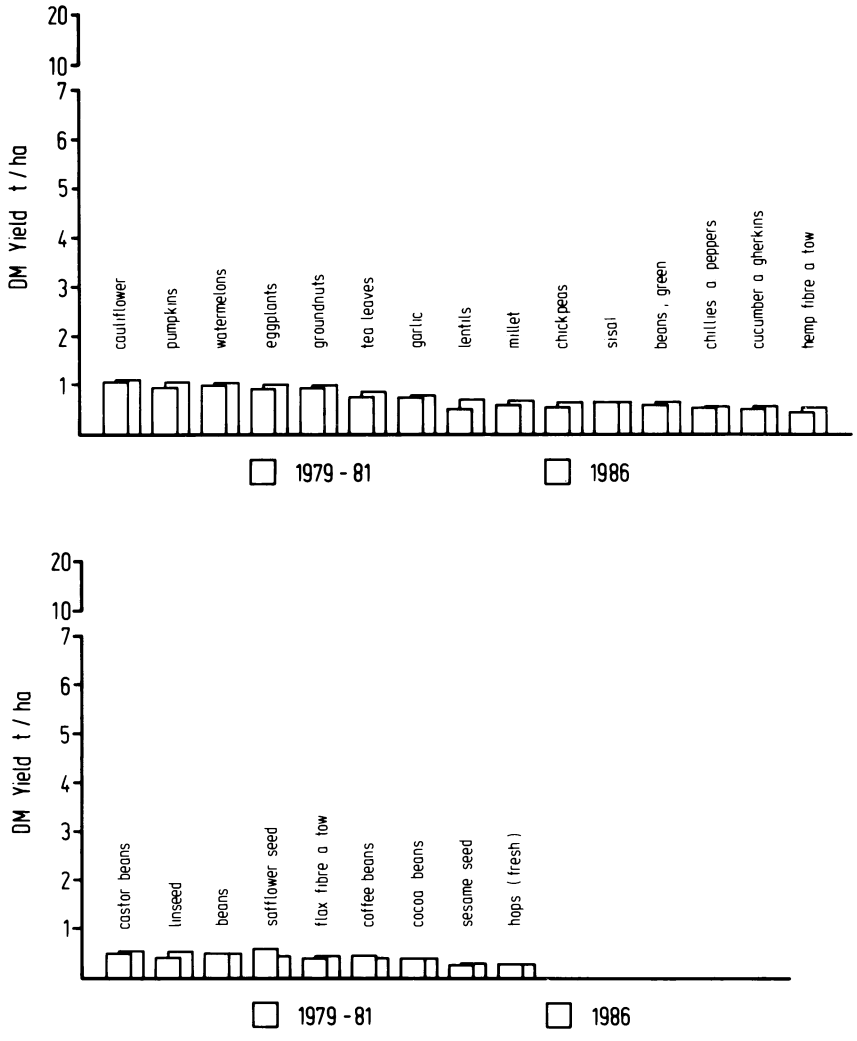


Fig. 1. 1–4. World dry matter average yield, tons per hectare (FAO 1987).

innovation. The genetic improvement of cultivars concerns mainly a more favourable dry matter distribution towards organs to be harvested (seeds, tubers, *etc.*) and to a lesser extent an increase of total biomass production (Spiertz, 1982).

The specific productivity and yielding ability in cereals may be appropriate to use it as an illustration since it is certainly an important breeding objective.

The contribution of genetic improvement in dry matter distribution to increased grain yield of *e.g.* winter wheat comparison of old and new varieties when grown under the same conditions shows that at the same time there has been only a small increase in total biomass production (Austin *et al.*, 1980). The same conclusion may be derived from data of De Vos and Sinke (1981) showing a rise in harvest-index from 0.37 to 0.49 and only minor differences in total biomass, nitrogen yield and nitrogen harvest-index (Table 1). Only in recent years has it become possible to measure the physiological component of the genetic improve-



ment in yield cereals. Experiments carried out at Cambridge showed that the modern varieties gave 60% more grain than the very old ones. and 6% more biomass (total above ground dry matter) despite being much shorter. Ear emergence occurred 8.5 days earlier in the modern than in the very old varieties and the corresponding difference in days to anthesis was 5.7 days (Austin, 1988).

Results from trials with old and new cultivars showed that in percentage terms the yield advantage of the modern varieties over the old ones was similar, but at all levels of nitrogen the new cultivar yielded more grain than the old cultivar. The yield advantage was close to 40%, irrespective of the amount of nitrogen applied. From the data of total above ground dry matter (biomass), no consistent difference between the varieties at any level of nitrogen could be recognized. The results also showed no differences between varieties in the total nitrogen content of the biomass. However, the nitrogen contained in the grain (grain yield × % N/100) was greater for the new cultivar than for the old cultivar at every nitrogen level, by an average of 10% (Austin, 1988).

Low input cultivars

Breeders have wished to see their cultivars succeed under the highest input systems and have

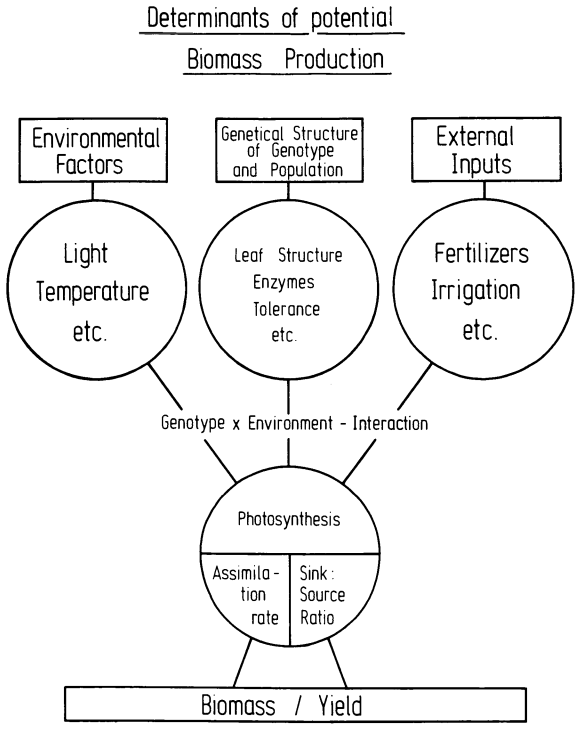


Fig. 2. Factors influencing crop productivity (El Bassam, 1985).

selected under these conditions, anticipating a continuation of the trend to intensification. There is evidence that under such conditions yields have become less variable (as measured by the interannual co-efficient of variation of yield) than formerly (Austin and Arnold, 1985), suggesting that modern agro-ecosystems provide a more stable environment for crops. Additionally, in selecting for wide adaptation and stability, breeders may have produced cultivars that are less vulnerable to climatic and other sources of variation in the agro-ecosystem. Breeding for an improved nitrogen utilization recently gained more interest because of economical and ecological problems caused by the increased use of fertilizers. The identification, selection and utilization of 'low-input' genotypes is an approach among several to develop low nutrient and low water consumptive genotypes with a high natural synthesis capacity.

The development of varieties with high nutrient exploitation capacity of soil resources is desirable and needed; these varieties might not be considered as 'low input varieties' as more nutrient will be removed and replenishment of the nutrient should be the consequences.

Low input varieties might be developed by selection and breeding and adapted for effective use of growth promoting factors in the local habitat such as light, natural fertility and compatibility with growth inhibiting factors such as drought, nutrient deficiency or salinity in soils. They have to possess high nutrient utilization efficiency, as a result of modified and improved physiological characteristics, *e.g.* high photosynthetic capacity, less losses of assimilate through respiration, adequate transport and distribution of assimilates within the plants (El Bassam, 1986).

Genotypic response to nutrients and water

As Vose (1987) noted, cultivars may be 'efficient' or 'inefficient' for uptake or translocation, or 'accumulators' or 'non-accumulators' of certain elements. Most approaches used to characterize the relationships between nutrient inputs and cultivars can be summarized as follows: Harvest-Index, Nitrogen Harvest-Index (Vose, 1987); Mineral Uptake Efficiency Radio (ER) = milligrams of dry shoot weight produced per milligram of elements in the shoot; Utilization Root (mg TDW produced per mg nutrient absorbed (Gerlof and Gabelman,

Table 1. Yield characteristics of five winter wheat cultivars

Variety and year of introduction		Total biomass minus roots ($t\,ha^{-1}$)	Grain yield (15% moisture) ($kg\,m^{-2} \times 10^{-2}$)	Harvest index (DM) ($g\,g^{-1}$)	Nitrogen yield ($g\,m^{-2}$)	Harvest index (N) ($g\,g^{-1}$)
Staring	(1941)	18.1	79.0	0.37	21.2	0.73
Felix	(1958)	17.4	87.8	0.43	19.5	0.78
Manella	(1964)	16.2	89.4	0.47	19.7	0.79
Arminda	(1977)	16.8	96.9	0.49	20.7	0.82
Hobbit	(1975)	17.7	101.8	0.49	21.3	0.79

(Based on data of De Vos and Sinke, 1981).

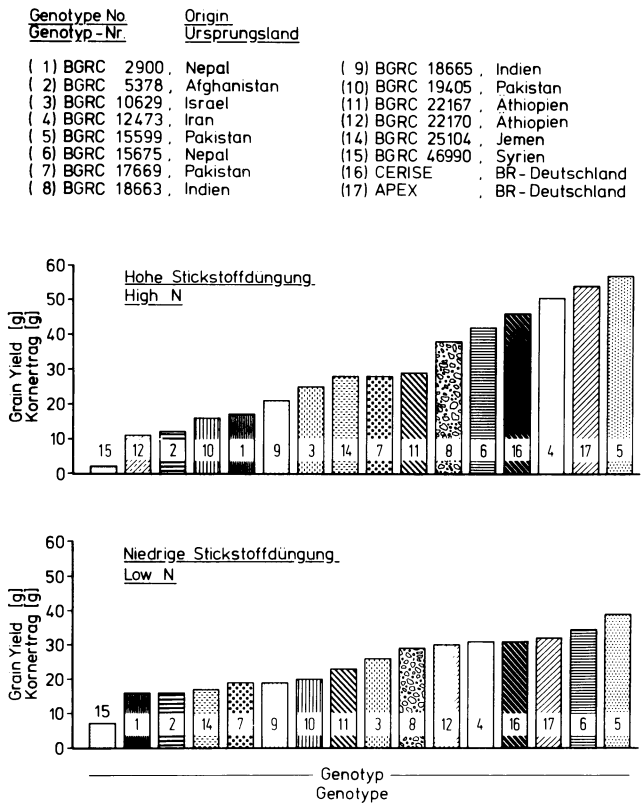


Fig. 3. Grain yield of different genotypes of barley under 2 nitrogen levels (El Bassam, 1986).

1985); Nitrogen Use Index (NUI) = the relation of grain N yield of a variety at certain N application to the sum of N in the grain at zero N-level plus total mineral nitrogen.

Klimashevsky (1986) used the expressions 'The Agrochemically Promising Cultivars and Coefficient of Absorption and Utilization of Fertilizers'.

An experimental set up was used to investigate the interaction effects between genotype, nutrients and water inputs. Worldwide germplasm of summer barley has been collected and representative accessions have been selected and included in the investigations according to the geographical origins and the breeding development stages, *i.e.* wild species, primitive forms, landraces and modern cultivars. Greenhouse experiments were conducted with a special device for water supply and different nitrogen levels (El Bassam, 1989).

After the 3 leaf stage the nutrients were added and in each pot 6 plants were left to grow till maturation. The treatments and interactions are described in Figure 3. Some results (Fig. 3) of these investigations can be summarized in the following points:

Some genotypes (no. 2, 12, and 15) produced less grain material under high nutrient than low nutrient levels.

Cultivars (no. 16 and 17) responded remarkably positively to nutrient supply. Some other genotypes (no. 4 and 5) were also very responsive to gertilizers.

The effect of fertilizers under dry soil conditions was very limited.

All genotypes produce less biomass under low water supply.

One genotype (no. 5) produced the highest grain yield under all cinditions.

One genotype (No. 3) indicated high yield stability.

The genotypes no. 4, 5, 6, 16 and 17 produced in all cases the highest grain yields.

Table 2. Yield characteristics and utilization efficiency in summer barley

Genotypes	Grain yield (g)	Harvest index	N-Harvest index	NUE	
				Grain	Straw
Landraces[a]	15.0	0.31	0.64	34	71
Modern varieties[b]	45.0	0.46	0.68	43	112

[a] n = 9.
[b] n = 4.

The yield level of all modern new varieties but only a few land races was high under low inputs. These genotypes were also very responsive to nitrogen and some genotypes showed high yield stability.

Comparison of the different genotypes when grown under the same conditions shows that an average rise in harvest-index from 0.31 of the landraces to 0.46 of the modern cultivars could be recognized. The data in Table 2 indicate that despite taking up similar amounts of nitrogen, genotypes differ significntly in biomass production and thus nitrogen use efficiency.

The present cultivars showed higher efficienty rates than the landraces or primitive and wild forms. Figure 4 illustrates an assumption for the results achieved; the present modern cultivars and some of the landraces possess high yield potential also under low levels of input and the response of these cultivars to increased inputs is wider and steeper. Also a wide range in the utilization efficiencies exists within the modern varieties.

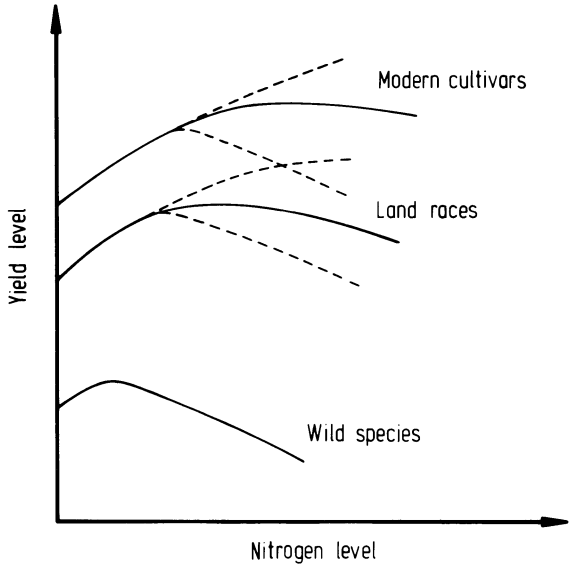


Fig. 4. Genotypic response of cereals to nitrogen supply (El Bassam, 1989).

A 3 year field experiment carried out with 15 new cultivars of winter wheat at 7 locations in Bavaria (Fischbeck, 1988) showed that differences between cultivars in overall yield potential are also seen under low input treatments of nitrogen and cultivars with higher yield potential are more affected by input reductions than cultivars with lower yield potential.

Conclusions

Prehistoric man could live from the abundance surrounding him. Present day man, with his limited resources, cannot exist without directing nature's evolution. Plant breeding is the act of developing cultivars for man and a tool among others to ensure his food supply. The basic process is selection among genetically different plants, nutrition and recombination.

Nevertheless, plant breeding should be prepared to face the challenges in the environmental constraints. The development of a new cultivar requires several years (6–15 years). Therefore, a clear and early formulation of new breeding objectives *e.g.* is essential. Several activities have been initiated to select cultivars of low input features and adequate nutrient efficiencies. Screening, evaluation and selection are essential steps for utilization of plant genetic resources as a tool to reduce the inputs and to protect the environment.

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Session 1

Physiological and biochemical mechanisms associated with genetic variation in utilization of a) nitrogen, b) phosphorus and c) other major nutrients

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 11–17.
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PLSO PN 123

Factors affecting the nutrient efficiency of plants

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Key words: fertilizer utilization, low input varieties, nutrient efficiency, rhizosphere, root morphology, root functions, varietal differences

Abstract

Shortage of resources and the environmental problems of intensive agriculture form the economic and ecological background of searching for more nutrient efficient plants. Breeding for nutritional efficiency, however, is still hampered by the lack of reliable screening criteria. Therefore, there is a necessity to define properties and metabolic activities of significance to nutrient efficiency. The following aspects will be discussed, paying special attention to areas for further research:

1. Root parameters related to nutrient uptake: Morphological root properties and physiological functions which may influence nutrient uptake are now reasonably well understood. However, work is still needed to evaluate the significance of root parameters under limited nutrient supply and to solve problems encountered in their determination.

2. Shoot-root relations: Nutrient uptake by the roots, their translocation to the shoots and subsequent redistribution among plant organs are governed by a complex exchange of substrates and information between shoots and roots.

3. Root-soil interactions: Topics of special interest are the root products affecting soil nutrient availability, the interaction between root exudates and microbial nutrient turnover, and the root enzymes related to the availability of organically bound nutrients in the rhizosphere.

Introduction: The need for more nutrient efficient plants

World population is still increasing and so is also the world food demand, mainly in the developing countries where the proportion of less fertile (*i.e.* semiarid, saline and calcareous) soils is particularly high. Problems of cultivation (*e.g.* soil erosion or adjusting irrigation and drainage to the water quality and to local conditions), form an additional threat there to soil fertility. Nevertheless, the local food situation just enforces the cropping even of low fertility soils. High yielding crops are therefore required there, but often they fail due to the difficulty to fulfill their nutritional requirements.

This cannot be overcome merely by fertilization, either because of the loss or the rapidly decreasing availability of supplied plant nutrients. An example for this is the phosphorus and micronutrient fixation in calcareous and alkaline soils. Serious nitrogen problems are also most common in semiarid soils due to their lack of organic matter.

In developed countries, on the other hand, high crop yields and related criteria (*e.g.* pest resistance) have been the major concern of plant breeders in the past. The soil nutrient levels necessary for verifying plant yield potentials were only of secondary importance. It was almost self-understood that agronomists could adjust their soils accordingly, mainly by ample fertilization.

A serious consequence of this concept, however, is the nowadays relatively low efficiency of fertilizers. The utilization of applied nitrogen, for instance, has often been found to be as low as 50% (Craswell and Godwin, 1984), and the recovery of phosphorus can be even lower. Low fertilizer

utilization increases the potential hazards of water and air pollution. Accordingly, improved nutrient efficiency is also a prerequisite of environmental protection.

Breeding for nutrient efficiency

The genetic dependency of nutrient utilization by plants has already been recognized more than half a century ago (Smith, 1934). In spite of considerable research work, however, (Barber, 1984; Clark, 1983; Gabelman and Gerloff, 1983; Graham, 1984; Loughman *et al.*, 1983; Mengel, 1983; Nielsen and Schjorring, 1983; Saric, 1983), it must be stated that, except for trace elements, nutrient efficiency (NE) is still not a common aim in crop improvement programmes.

Breeding for nutrient efficiency requires to

(1) establish a clear conceptual definition for NE
(2) clarify plant properties, shoot-root relations and root-soil interactions related to NE,
(3) develop screening techniques based on the main factors governing NE and
(4) develop and/or improve the appropriate methods for measuring NE.

Uptake efficiency and utilization efficiency

In this paper an agronomic definition of NE is used which relates plant productivity to nutrient supply (Craswell and Godwin, 1984):

$$NE = \frac{\text{plant yield}}{\text{nutrient supply}}$$

Unfortunately, the characterization of nutrient supply under field conditions has to face several uncertainties related to the loss of nutrients and the dependence of their availability on soils and climatic conditions as well as on water supply. Basic research on NE and related screening requires therefore a more simple rooting substrate. A standardizable substrate for NE investigations, however, has not yet been developed. Solution cultures are less adequate due to the lack of physical root-soil interactions as well as of nutrient mobilization from less soluble and organic fractions.

The definition of NE as plant yield per unit of nutrient supply results in its dependency on two interrelated groups of plant factors, namely a) plant properties related to the "uptake efficiency", which is nutrient uptake relative to its supply and b) factors related to "utilization efficiency", representing plant yield relative to nutrient uptake.

Plant and soil properties related to uptake efficiency

Understanding the processes involved in the uptake of nutrients from soils has increased greatly during the last decades. The most significant contributions to his progress were:

(1) the applications by Epstein and Hagen (1952) of enzyme kinetics to describe the dependence of nutrient uptake by roots on its concentration,

(2) the concept of Bray (1954) of nutrient mobility in soils, describing their transport to the roots, and its further elaboration by Barber (1962) and Nye (1966),

(3) the incorporation of 1. and 2. into mathematical models simulating the nutrient uptake by growing roots from soil (review: Barber, 1984), which in comparison to actual measurements enables to evaluate the adequacy of the parameters used, and

(4) rhizosphere research clarifying the efffect of plant roots on nutrient availability (*e.g.* Marschner *et al.*, 1986, Helal and Sauerbeck, 1987c).

These achievements form the basis for an identification and quantification of both the plant and the soil factors involved. The most important parameters are summarized below:

Plant and soil parameters related to uptake of nutrients by growing roots

a) *soil parameters*

1. C_l = soil solution concentration
2. b = buffering power of the soil solution by nutrients associated with the solid phase
3. D_e = effective diffusion coefficient

b) *plant parameters*

4. v = rate of water influx into the root
5. L = total root length

6. r = mean root radius
7. k = root growth rate
8. I = influx rate of nutrient at a given concentration
9. α = absorption power, influx rate relative to concentration
10. I_{max} = maximal influx rate if C_l is not limiting
11. K_m = Michaelis coefficient = C_l necessary to drive I to 1/2 I_{max}
12. E = efflux rate = back diffusion of nutrients from the root to the rhizosphere
13. C_{min} = soil solution concentration at which I = E = no net influx

No. 1–4 determine the rate of external nutrient transport to the roots. No. 5–7 are morphological root characteristics related to the root surface area and its change with time. No. 8–11 describe the dependency of nutrient influx into the root on its concentration in the soil solution. No. 12 and 13 finally express the back diffusion of nutrients from the root to rhizosphere.

Significance of individual root parameters

The significance of the afore mentioned parameters depends on the mobility and supply of a nutrient relative to plant demand. Under ample supply (high C_l) of mobile nutrients such as calcium and nitrate, a significant depletion of the rhizosphere is not expected. Under such conditions the capacity of the uptake mechanism (I_{max}) rather than its affinity (K_m) will be of primary significance.

In the case of less mobile nutrients with low C_l and D_e and high b values such as phosphorus and potassium, the transport in soil to the root surface is often limiting, which results in a depletion of the rhizosphere (Hendriks *et al.*, 1981). Under such circumstances three other groups of factors are of significance, namely

- the parameters L and r which are related to root surface area, since longer and finer roots mean that the soil depletion per unit of root surface will be lower,
- the affinity of the uptake mechanism K_m and C_{min} (*i.e.* its effectiveness in the low concentration range) and
- the modification of the diffusion controlling soil parameters C_l and D_e by plant root activities as will be discussed below.

For estimating the relative significance of these individual parameters two approaches proved to be useful:

(1) sensitivity analysis of simulated uptake to variation of the parameters used in mathematical models (*e.g.* Silberbush and Barber, 1983),

(2) comparative studies relating nutrient uptake by plant varieties to their root parameters (*e.g.* Nielsen, 1983; Barber, 1984).

The genetic variability of such root parameters is now well established. However this has not yet found much attention in breeding for NE. Possible reasons for this may be the problems encountered in the determination of root parameters, their modification by soil conditions and their dependence on shoot activity.

A very important problem in root research is estimating the functionally active part of the root system, which may be much smaller than the total root length values measured conventionally (Ward *et al.*, 1978). This corresponds with the fact that the apical region is much more active in exudation (Rovira, 1969) and in iron mobilization (Römheld and Marschner, 1983), than the basal root parts are.

Shoot-root relationships affecting nutrient efficiency

A growing plant tissue is a sink for both photosynthetic products and mineral nutrients. The balance between these two streams is still poorly understood (Clarkson and Hanson, 1980). However, it is now clear that the three main processes, uptake of nutrients by the roots, their translocation to the shoots and subsequent redistribution among plant organs are governed by complex communications between shoots and roots. A model for this shoot-root co-operation has been proposed by Cram and Pitman (1972). According to this concept the concentration of ions and energy-providing substrates as well as the level of hormones are important factors in a feedback system coordinating root activity with shoot demand.

As an example, ample energy supply from the shoots (Sauerbeck and Johnen, 1976) is essential for the development and functional integrity of plant roots. This means that not only root growth but also its physiological activity is under shoot control (Pearson and Steer, 1977).

There is also a considerable exchange of phytohormones from shoots to roots, which may affect nutrient uptake in a rather complex way (Lüttge and Higinbotham, 1979). Similarly cytokinins produced in the root tips are transported acropetally (Torrey, 1976) to the shoot where they affect leaf senescence and nutrient redistribution in the plant. All these aspect ought to be clarified before the variability of related root parameters can be understood.

Root-soil interactions

Soil conditions affect root activities directly or indirectly in a most complex way. Physiological root functions for instance are especially sensitive to variations in soil temperature and aeration. Generally, however, almost all chemical and physical soil factors influence root development and related activities.

An example (Sauerbeck and Helal, 1986) of the effect of soil bulk density on the morphology and assimilate consumption of maize roots is given in Table 1. These results indicate that even a relatively small increase in soil density already affects the total root length, the number of individual root apices and the substrate consumption by the root. The need for standard conditions, including soil density, for root screening is therefore evident.

One of the most intricate aspects in this field is the modification of nutrient availability in soils by plant roots. Such root influences on soil were already anticipated in the last century. As early as 1839 Sprengel pointed out that plants can prepare part of their food by releasing acids which dissolve otherwise insoluble minerals. After a long stagnation, considerable progress in rhizosphere research (Marschner *et al.*, 1986) was made possible by the introduction of new experimental techniques (review by Helal and Sauerbeck, 1987c). These include

(1) homogeneous labelling of plants with ^{14}C to quantify their root turnover (Sauerbeck and Johnen, 1976).

(2) improved methods for exudate analysis (*e.g.* Moghimi *et al.*, 1978) and

(3) for sampling rhizosphere soil (Helal and Sauerbeck 1983) as well as

(4) for determination of the rhizosphere microbial biomass (Jenkinson and Powlson, 1976).

(5) *in situ* measurement of rhizosphere pH (Marschner and Römheld, 1983).

These techniques now enable investigating the root activities and rhizosphere processes related to NE.

Root exudates include a great variety of substances (review by Curl and Truelove, 1986) which affect nutrient availability in various ways. Some examples are summarized in Table 2 and may be grouped as follows:

(1) direct influences on nutrient solubility, transformations and binding in the rhizosphere soil (No. 1–4) and

(2) indirect influences through affecting microbial activity (No. 5).

Most efforts were so far devoted to direct root effects on the uptake efficiency of trace elements (Helal, 1988; Marschner, 1989; Römheld and Marschner, 1986). Information about root influences on turnover and availability of major nutrients is, however, still rather scarce. More research work is, therefore, required to clarify the mechanisms related to:

(1) the denitrification-losses in the rhizosphere (*e.g.* Smith and Tiedje, 1979),

(2) the mobilization of unexchangeable K by roots (*e.g.* Steffens and Mengel, 1979) and

(3) the mobilization of inorganic and organic soil phosphorus (*e.g.* Helal and Sauerbeck, 1987b) by roots and root-microbial association.

For the mobilization of soil inorganic phosphorus in the rhizosphere, for instance, three different mechanisms have been suggested, namely

(1) the acidification of the rhizosphere (Hedley *et al.*, 1982, Nye and Kirk, 1987).

Table 1. Morphological properties and photosynthate consumption of 23 d old maize roots depending on soil compaction

Soil bulk density	1.2	1.4	1.6	$g\,cm^{-3}$
Number of individual roots	26.1	15.6	12.0	$\times 10^3$
Total root length	246	125	89	m
C consumption per meter root	4.3	6.1	7.2	mg

(after Sauerbeck and Helal, 1986).

Table 2. Root activities and products affecting nutrient availability in the rhizosphere

Root activity	Nutritional effect	References (examples)
1. Acidification of the rhizosphere	Solubility of Ca, P, Al and trace elements, microbial and enzyme activity	Marschner and Römheld, 1983; Nye and Kirk, 1987
2. Organic acids and chelators	Solubility of Ca and P, transport of trace elements, microbial interactions	Gardner *et al.*, 1983, Römheld and Marschner, 1983
3. Reductants and oxidants	Fe- and P-solubility, N-turnover, microbial interactions	Brown and Ambler, 1973; Ando *et al.*, 1983
4. Extracellular enzymes	Turnover of organically bound nutrients, redox reactions	Chang and Bandurski, 1973; Helal and Sauerbeck, 1987a
5. Substrate for microbial biomass	Microbial nutrient turnover, hormone production	Brown, 1972; Helal and Sauerbeck, 1986

Table 3. Increase of microbial biomass in planted soil and contribution of plant C to microbial growth in soil zones differing in proximity to maize roots (initial biomass content = 12 mg ^{12}C/zone)

Soil zone	Microb. biomass C after planting		Microb. biomass C increase during planting	
	mg C	(% of initial)	Total mg C	Plant-derived (% of total)
Root zone	59	(492)	47	(82)
Adjacent zone	26	(217)	14	(44)
Distant zone	22	(183)	10	(35)
Sum	107	(297)	71	(68)

(after Helal and Sauerbeck, 1986).

(2) the exudation of citric acid (Gardner *et al.*, 1983) and

(3) the rhizosphere microbial activity (Helal and Sauerbeck, 1985).

The action of the latter is based on:

(1) the considerable increase of microbial biomass found in the rhizosphere (Table 3, Helal and Sauerbeck, 1986),

(2) the occurrence in the rhizosphere of species which are effective in desorbing inorganic phosphorus (Louw, 1970),

Table 4. Concentration of inorganic (P_i) and organic (P_o) phosphorus in soil solution of soil (luvisol) zones differing in proximity to roots 28 days after planting with maize

Soil zone	P_i (ppm)	P_o (ppm)	P_o/P_i
Control (unplanted)	0.15	0.43	3
Root zone	0.12	0.64[a]	5
Adjacent zone	0.18	1.36[b]	7
Distant zone	0.16	0.58	4

[a] Significantly different from the control at $P0.05$.
[b] Significantly different from the control at $P0.01$.
(after Helal and Sauerbeck, 1987b).

(3) the microbial production of soluble organic phosphates which are much more mobile than the inorganic P-forms in soil (Table 4, Helal and Sauerbeck, 1987b) and

(4) the very high phosphatase activity of intact plant roots (Helal and Sauerbeck, 1987a).

Recent work (Helal, 1988, this volume) has for instance demonstrated considerable varietal differences in both root phosphatase activity and the ability of bean plants to utilize organic phosphates. Further research will be necessary to identify rhizosphere microorganisms which are efficient in P-desorption, and to clarify the factors controlling their abundance. Similarly the significance of root phosphatase activity as a criterion of NE awaits its evaluation under field conditions with limited mineral phosphorus supply.

Conclusions

On the basis of the problems discussed, areas for further research may be identified as:

(1) quantitative determination of the most important root parameters.

(2) characterization of rhizosphere mechanisms related to the availability of major nutrients, and

(3) clarification of the shoot-root interactions governing the activity and longevity of roots as well as the redistribution of nutrients in the shoots.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 19–23.
PLSO PN 124
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Physiological basis of genotypic plant distinctions in mineral nutrition

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Key words: absorption, breeding, efficiency, genetics, nutrients, physiology, roots, translocation

Abstract

Some results of research carried out at the Department of Physiology and Genetics of Plant Mineral Nutrition are presented with special reference to genetically determined differences between plant genotypes in nutrient absorption and utilization efficiency per yield unit. Attention is focussed upon root function under NPK excess or deficiency. The role of genetics of mineral nutrition as a basis for target-oriented engineering of agrochemically promising (energy-saving) varieties (APVs) is stressed.

Introduction

Fertilizer application is one of the key factors of crop yield increase. But, in the development of energy-saving technologies in plant industry, one should strive to raise the plants' coefficients of absorption and utilization of fertilizers (UFC) (Klimashevsky, 1964; 1974; 1985; 1986). This is possible only by developing a system of fertilizers that takes into account genetically determined specificity of the cultivar–fertilizer interaction effect (Chernyshova, 1976; Klimashevsky, 1974, 1984; 1985; 1986; Klimashevsky and Chernyshova, 1982; Saric, 1981; 1987).

Economic view of the problem

More than 300 forms of wheat, barley, pea, soya-bean, buck-wheat, oat and potato were analysed as to their APV, *i.e.* the genotype ability to synthesize the maximum yield in terms of inputs of N, P and K absorbed per time unit (Klimashevsky, 1974; 1985). It is shown that APV boost yield by 97–118% on account of better NPK utilization, while the agrochemically inferior forms — by 22–29% only. The former had UFC as high as 32–46%, the latter 18–22%. UFC varied under the influence of biotic and abiotic environment factors. UFC of varieties more tolerant to stress show greater consistency over the years. Unit cost of additional yield on account of NPK use was 2.9 times lower than that for non-APVs (Klimashevsky, 1974; 1983; 1985; Klimashevsky and Chernyshova, 1980).

Growth intensity, plant anatomy and morphology

Cultivars with more rapid development have a high index of organic matter synthesis per time unit and a greater response to BPK (Klimashevsky and Chernyshova, 1982). Photosynthetic reaction rates depend upon the translocation rate of photosynthetic products to the areas of their utilization. APVs should therefore have a more extensive vascular system. Thus, a winter wheat variety highly responsive to NPK, if compared with Novoukrainka, has a much greater number of fibrovascular bundles, more developed xylem and phloem, greater area of cells surrounding the central vascular bundle in the flag and the last but one (penultimate) leaves. In APVs of oat the stem diameter and the number of segments in sieve tubes are much larger than in those with a weak NPK response (Honsley and Peterson, 1982; Klimashevsky, 1985; Volodarsky and Tsiunovich, 1979). It is shown that under different conditions of mineral nutrition the number of grains ($r = 0.80, 0.66, 0.77$,

0.81) and the grain yield per panicle (r = 0.75, 0.55, 0.74, 0.79) can be predicted based on the stem diameter, the number of bundles, their area and the phloem area, respectively.

Representatives of *Triticum* with different ploidy levels and genome structures have different veinlet patterns, which has a bearing not only on assimilate translocation, but, consequently, on the genotype response to NPK (Klimashevsky, 1985a,b). Rice cultivars with a high degree of response to nitrogen have short and strong straws; the divergence angle between leaf and stem in these varieties is more acute (Klimashevsky, 1974). In APVs of winter wheat the average grain/straw ratio is 1.13, in the varieties with a lower response to nitrogen — 0.56.

There is close correlation between the genotype response to NPK and Si content in spikelet scales (SS) and flag leaf (FL) tissues for wheat and barley plants (Klimashevsky, 1981). Moskovskaya 35 and Sete Cerros 66 wheat cultivars and Moskovskiy and Nadya barley cultivars, producing more yield per unit of absorbed nutrients, contained the averages of 3.5 and 4.6 of Si in SS, and 2.4 and 3.9% in FL tissues, respectively. The corresponding values for cultivars with a poor response to NPK (Diamant and Birysinka wheats, Varde and Winer barleys) were 0.9 and 1.4, 0.7 and 1.1%. Si content in SS of the varieties under study decreased with increase of nitrogen dosage from 0 to 150 kg/ha. Varieties more responsive to nitrogen fertilization (150 kg/ha) decreased their Si content by 26%, while those agrochemically unpromising — by a factor of 3.8. It is noteworthy that Si content in SS and FL of wheat and barley is not only a sensitive indicator for adaptation of plants to high N levels, but also is practically unvarying in soil conditions with changing water content. The correlation between N-responsiveness and Si content in SS is 0.85 for wheat and 0.91 for barley.

Specificity of root physiological activity

Genotypic specificity of root nutrition manifests itself in ion absorption and assimilation processes (Klimashevsky, 1964; 1974; 1984a; b; c; 1986; Klimashevsky and Chernyshova, 1982a; b; 1983). Root-to-shoot mass ratio is determined by exchange of metabolites between organs, phytohormone regulatory function and specificity of source-sink relations of chlorophyllous and nutrient-absorbing organs (Chernyshova, 1984; Klimashevskaya, 1984; 1986).

An APV is characterized by the quantity of absorbed nutrients per unit of absorbing root surface, as well as by the quantity of organic matter per time unit, calculated per unit of NPK consumed. This is a measure of the rationality of genotype-fertilizer interaction (Klimashevsky, 1974). Studies of many cultivars, hydrids and mutants of a number of crops found that genotype response to NPK depends on root size rather than on their consuming and metabolic activity (Klimashevsky, 1974). APVs' features are: first, economical N and P expenditure for grain unit formation, and second, root productivity index (shoot or grain weight-root weight or its effective absorbing surface). Genotype response to N often correlates with nitrate reductase activity, and that to P — with acid phosphatase activity (Klimashevsky and Chernyshova, 1982b; 1983), as well as with the root cation exchange capability (CEC) (Klimashevsky, 1964; 1974). Taking into consideration that the root CEC correlates with nitrogen content and the total cation content, it can be assumed that it is an important nutritional factor determining the specific ion absorption. The root CEC may be considered also ecologically. Differential distribution of perennial grasses in climatic regions can be accounted for by the relation between the root CEC and cation absorption. This is of great importance for compilation of programmes for plant cultivation.

Let us consider one of the physiological features typical of APVs. It is known that the contact area of root and soil is determined by root hairs (RH). Many cereal groups have so specific amorphology of RH cells, that it can be used as an intraspecific criterion (Esau, 1969).

It is shown that the RH length and their number on the root surface as well as the protein nitrogen content in the root differ between cultivars (Klimashevsky and Tokarev, 1980). Thus, the average number of RH in highly responsive forms of maize, barley and wheat is 223, the average length being 420 μm, protein nitrogen content 3.13 μm, and for those with poor response — 100 and 267 μm and 2.21 μg, respectively. The RH length and N_{prot} content in root correlated with the total yield and response to root nutrition. When comparing kinetics of P absorption by the RH area it

was found that wheat genotypes with a higher RH index absorbed by 30–40% more P than those with a small RH area. Different *Trifolium* and *Medicago* species with a higher P absorption have more numerous RH. In this case interspecific differences in P absorption and organic matter accumulation are four–sixfold.

This observations were proved by specific examples of 12 wheat, 8 barley, 10 tomato and 4 aubergine cultivars; correlation between RH and P absorption (^{32}P test) was 0.724. The differences in P absorption can be accounted for not only by both RH quality and their longevity but also by the root excretory activity as well. Activity of acid phosphatase in P-efficient plants having more numerous RH when the soil solution has a great shortage of P, is 2.2 times higher than in the case of varieties with a small RH number per root surface unit. The downward flow of sugars from photosynthetic organs and utilization rate of ^{14}C-assimilates from root cells in wheat varieties with a greater number of RH were also higher.

One of the possible approaches to the understanding of the genetic nature of mineral nutrition is the reciprocal grafting method. The method allows us to assess the role of the absorbing organs. Experiments with tomato plants (Volghskiy 288 and Volgogradskiy 5/95, with a low and a high number of RH, respectively) showed that in the case of grafting of the first variety shoot on the second variety root, ^{32}P absorption was increased by 50–60% as compared with the reciprocal case. F_2 plants, the product of crossing of Volgogradskiy 5/95 (mother) and another cultivar with the minimum RH number and poor ^{32}P absorption, also had a substantially higher P-absorption capacity.

One of the main characteristics of RH cells is a great number of mitochondria and dictyosomes that have a structure typical of a physiologically active cell. The morphology of the cells reflects their high activity (energetic substrates synthesis, absorption and translocation, and membrane building). The Golgi apparatus in hair cells is in the hypersecretion state (see Atlas, 1972). This is an evidence of RH properties being the basis for the fairly clear differences observed under P-deficient conditions. The mitochondria number in RH cells of Volgogradskiy 5/95 tomato was 2.9 times higher, the ATP content in mitochondria suspension was 1.6 times as high as that in P-inefficient varieties. The mitochondria functional activity (phosphorylation rate and O_2 absorption) was also (130 and 180%) higher than that in other varieties (Klimashevsky and Tokarev, 1988).

The above mentioned and other published results (Klimashevsky, 1974) show that there is a practical possibility to obtain breeding material with a significantly increased P-absorption capacity which is of value for conditions of mobile P deficiency as well as for target-oriented development of agrochemically superior (energy rational in P nutrition) genotypes. It is especially important for varieties growing on soils with increased Al^{3+} content (Klimashevsky and Chernyshova, 1983).

The results of studies of genetic variability in response to P nutrition, the leading role of the root in genetic control of nutrient utilization and response of plants to fertilization, the aspects of genetic control of the root absorption of nutrients, identification of plant forms with a favorable nutritional response, methods for evaluation of APVs, the principles of simulating of crop plants on the basis of the physiological characteristics of root systems as well as the evolutionary aspects of plant nutrition were published (see Klimashevsky). In our publications there is a detailed discussion of a number of cereal and bean crop cases of specificity in their high or low response to NPK. This specificity is attributed primarily not to the preliminary CO_2 fixation but rather to the nature and direction of post-photosynthetic processes and complicated secondary biosynthesis, and to peculiarities of enzyme functions directly participating in CO_2 assimilation in the course of dark photosynthetic reactions.

Conclusions

Numerous data testify to the fact that the unilateral breeding of plants directed to increase the latent productivity led to a decrease of the plant resistance to abiotic and biotic adverse factors; the changes of shoot-to-root ratio and a significant loss of synthetic and absorbing activity (in comparison with the wild types) resulted in low agricultural and energy potential. Moreover, plant breeders do not carry out target-oriented genetic programmes which could justify the cost of mineral fertilizers.

Uniform, or so called intensive forms of plants, which contributed to adoption of energy-consuming technologies in agriculture, appear productive only under the conditions of easily available mineral elements for root nutrition, application of herbicides, etc. Therefore, the task of a drastic decrease of 'power cost' we pay for agricultural output is very important when developing a rational plant industry system. This crucial part is the responsibility those working on the genetics of mineral nutrition and plant breeding. Preliminary calculations show that rational accumulation of indirect energy resources (the possibility of better utilization of solar radiation being excluded) is possible through systematic plant breeding of such forms, the cultivation of which will decrease demand for fertilizers by 30–40% and more.

Genetics of mineral nutrition is concerned with genetic variability, inheritance and genetic control of plant mineral nutrition factors, *i.e.* the process of efficient absorption and utilization of mineral elements as well as with resistance to stress factors in the root-inhabitated soil volume. The main objects of plant nutrition studies should be natural and induced genetic variability in mineral nutritional characteristics, diversity of genetic adaptation of plants to their mineral environment. A special attention in these studies should be paid to the analysis of possibilities of combining in one genotype systems which are highly efficient in terms of absorption and utilization of mineral substances without offsetting the balance in other physiological processes governing the production potential of the plant.

The development of mineral nutrition genetics as the scientific basis for target-oriented engineering of agricultural APVs, tolerant to ionic toxicity in the root area and to herbicides, will produce the principal component of emerging 'biological farming'.

Active search is also needed for donors to APVs among both crop plants and the wild types showing energy-saving absorption under high stress conditions of existence (Klimashevsky and Tokarev, 1988) as living systems often exist not only thanks to environment, but also in spite of it (Alexandrov, 1985).

Acknowledgements

The author would like to express his gratitude to all his colleagues who had helped with the research, particularly to N F Klimashevskaya. Special thanks to B I Tokarev and H P Losovskya for the English translation.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 25–31.
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PLSO PN 125

Concentrations of N, P, and K and dry matter mass in maize inbred lines

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Key words: dry matter, element, inbred line, maize

Abstract

Maize inbreds (Mo-17, ZPL-501, OH-43, A-632-Ht, ZPL-500) of different genetic origin and almost coincident growing season were selected. Plants were grown in nutrient solution of different nutrition levels (1/8 and 1/2 strength of a full Reid-Jork solution, full nutrient solution 1, and 2 and 3 times as strong as a full nutrient solution) for 25 days. The effects of mineral nutrition on dry mass of root and aboveground part and ion concentrations were examined.

Dry matter accumulation into root and aboveground part depended upon inbred line and the nutrition variant employed. On the average, the highest dry mass was obtained with ZPL-500 and ZPL-501, whereas the lowest with A-632-Ht. Significant differences in element concentration relative to concentration of nutrient solution were found. The smallest differences were recorded for N (0—30 %) while those for other elements were higher ranging from 40 to 70 %. The differences in element concentration were more expressed in aboveground parts than in root. On the average, concentrations of elements increased as that of nutrient solution increased.

Introduction

The relationship between plant genotype and the supply of mineral nutrients has been studied from various aspects by using different methodologies. The genetic-physiological and biochemical mechanisms resulting from this particular relationship however, are still poorly understood. Many of these processes are under genetic control, but may be altered dramatically when plants are grown in different nutrition conditions (Gabelman and Loughman, 1987; Sarić and Loughman, 1983). Available results indicate that there are differences between different maize genotypes in uptake and utilization of certain elements of mineral nutrition (Clark and Brown, 1974; Estes, 1976; Klimashevsky, 1966; Moors, 1922; Sarić *et al.*, 1974).

Materials and methods

Experiments used five maize inbreds (Mo-17, ZPL-501, OH-43, A-632-Ht, and ZPL-500) of different genetic origin and almost coincident growing season. Plants were grown in a nutrient solution of 1/8 and 1/2 strength of that described by Reid-Jork (1958), full nutrient solution (1), and 2 and 3 times as strong as the full nutrient solution, for 25 days. Dry mass of root and aboveground part and ion concentration were examined.

Dry matter mass was determined by drying at 105 °C. Analyses of elements were performed in three replications: N with the Kjeldahl method, P spectrophotometrically, and K by flame photometry. Data were processed statistically by the variance analysis (LSD-test).

Results

Dry matter mass

Significant and highly significant differences were obtained between inbreds in the dry mass of aboveground parts when individual nutrition variants were analyzed (Fig. 1). The smallest dif-

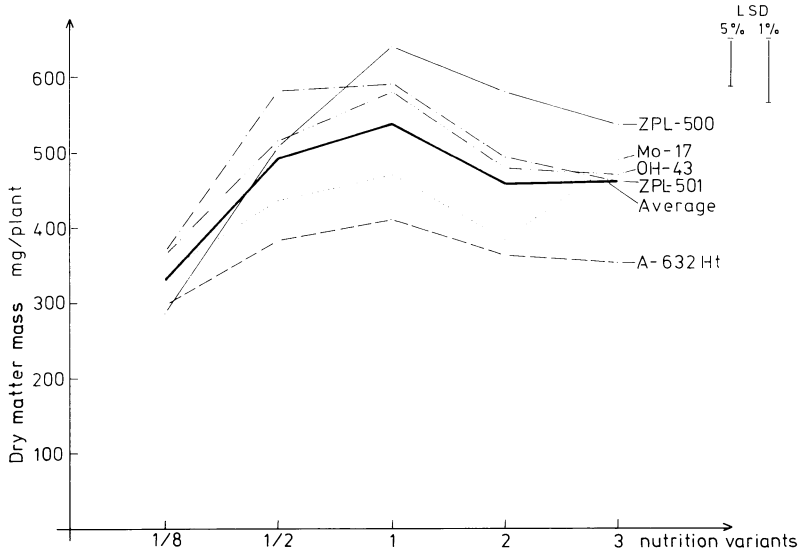

Fig. 1. Dry matter mass of aboveground parts in different inbred lines of maize.

ferences were recorded with 1/8 nutrition variant. The line ZPL-501 was characterized by a greater dry mass of aboveground parts at lower concentrations, whereas ZPL-500 at higher concentrations of mineral elements. The lowest dry mass of the aboveground part, irrespective of concentration of nutrient solution, was found in the line A-632-Ht. Averages for nutrition variants showed that the highest dry mass was obtained with the control plants (1), then 1/2, 2, and 3, whereas the lowest with 1/8, irrespective of inbred examined.

When dry mass of roots were compared (Fig. 2), also ZPL-501 and ZPL-500 showed higher values, whereas OH-43 and A-632-Ht were more inferior than averages which indicated that mineral nutrition certainly affected root mass, but the differences obtained were smaller than in aboveground parts.

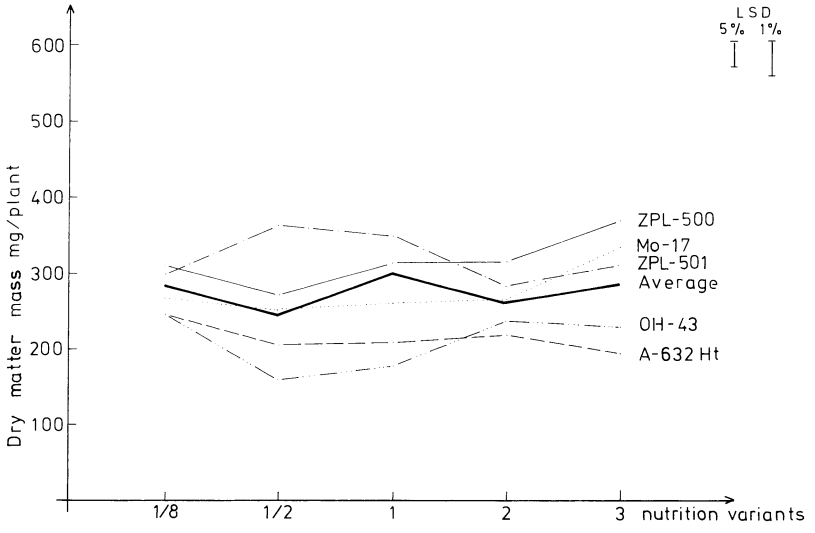

Fig. 2. Dry matter mass of roots in different inbred lines of maize.

Nitrogen concentration

Greater variations were found in aboveground parts (Fig. 3) than in roots (Fig. 4) and depended not only upon the characteristics of inbred lines but also upon the nutrition variant. The data obtained also show that the differences in nitrogen concentration of the aboveground part were considerably greater at lower concentrations of all elements (1/8 and 1/2) than in the remaining variants, and according to average values, as concentration increased nitrogen concentration in inbreds also increased.

On the contrary, root nitrogen concentration showed that the differences, although statistically significant, were smaller while the greatest difference was recorded in control plants. Root nitrogen concentration increased as concentration of all elements in nutrient solution increased.

Phosphorus concentration

The lines A-632-Ht and OH-43 showed increased concentrations, whereas ZPL-500 was characterized by a reduced concentration of this element

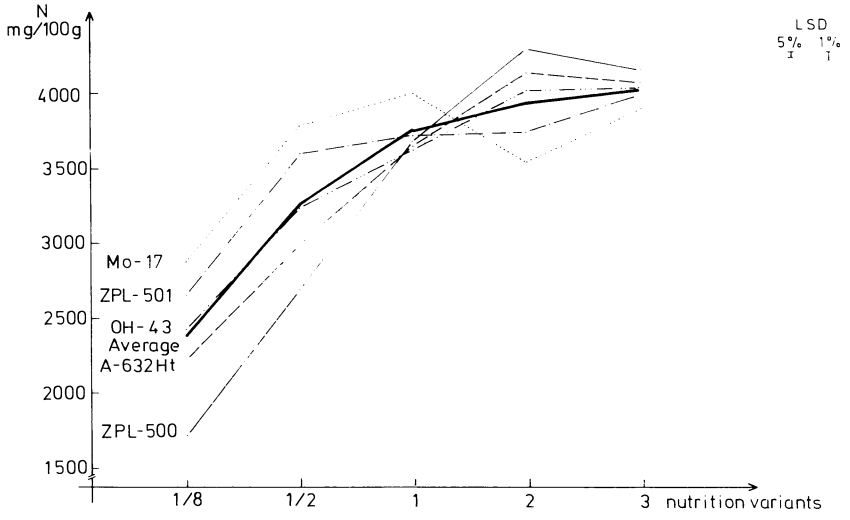

Fig. 3. Nitrogen concentration in aboveground parts of different inbred lines of maize.

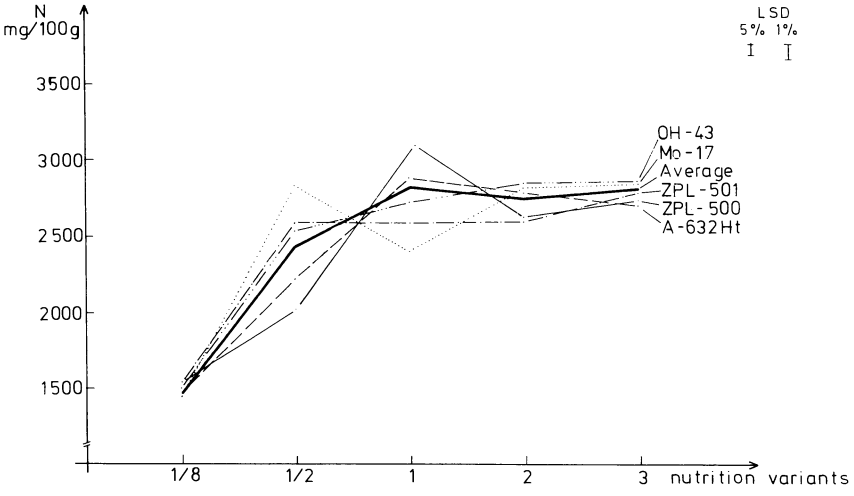

Fig. 4. Nitrogen concentration in roots of different inbred lines of maize.

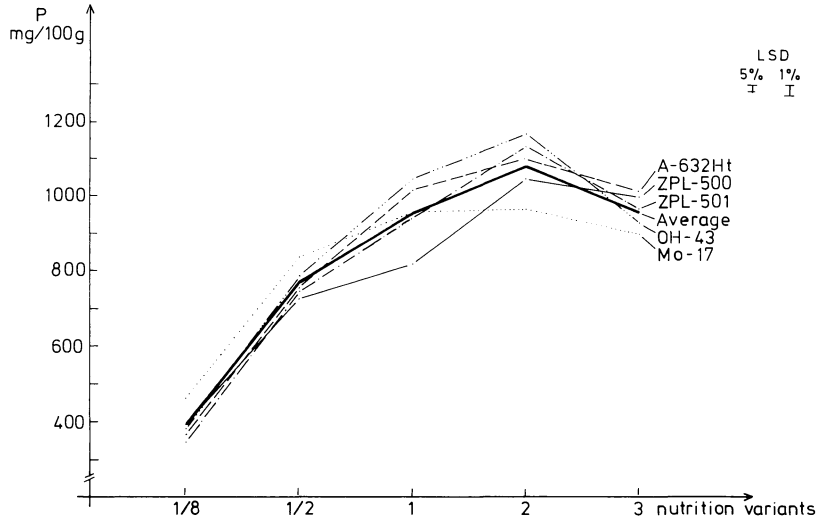

Fig. 5. Phosphorus concentration in aboveground parts of different inbred lines of maize.

in aboveground part (Fig. 5). When nutrition variants were analyzed, the highest concentration was obtained with double strength nutrient solution.

With regard to roots (Fig. 6), the differences in phosphorus concentration between inbreds were considerably greater with all the variants employed. The line Mo-17 showed an increase, whereas A-632-Ht had a reduced concentration of this element in root.

Potassium concentration

In contrast to nitrogen and phosphorus, the highest potassium concentration in the aboveground part was obtained with variant 1/2 (Fig. 7) while averages show that as the concentration of elements in the nutrient solution increased, potassium concentration in the aboveground part decreased. On the contrary, root potassium concentration (Fig. 8) increased as concentration of

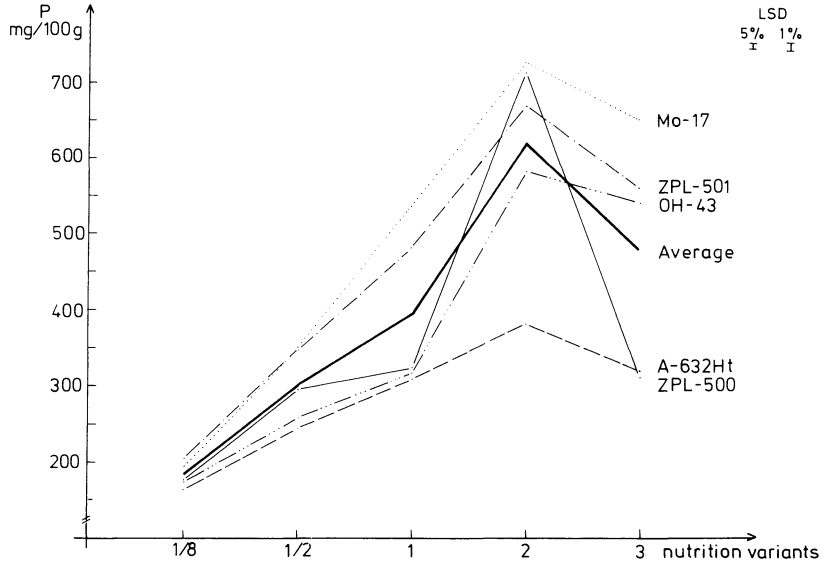

Fig. 6. Phosphorus concentration in roots of different inbred lines of maize.

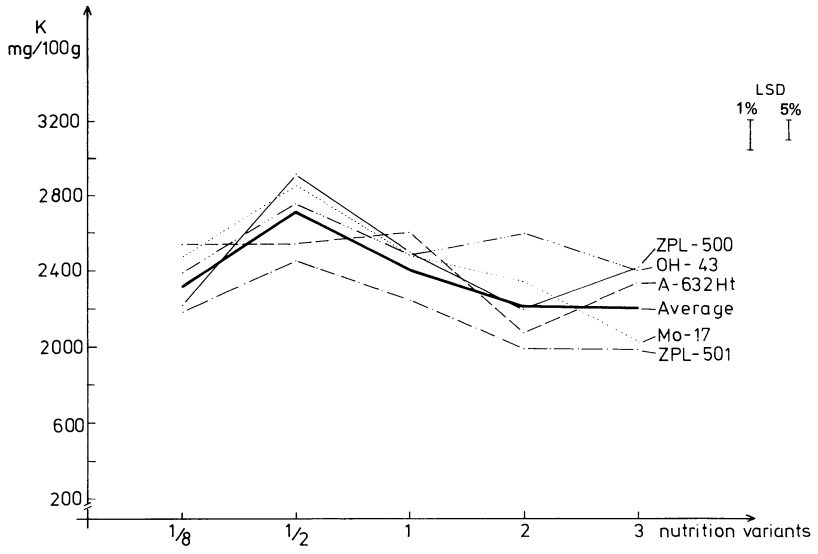

Fig. 7. Potassium concentration in aboveground parts of different inbred lines of maize.

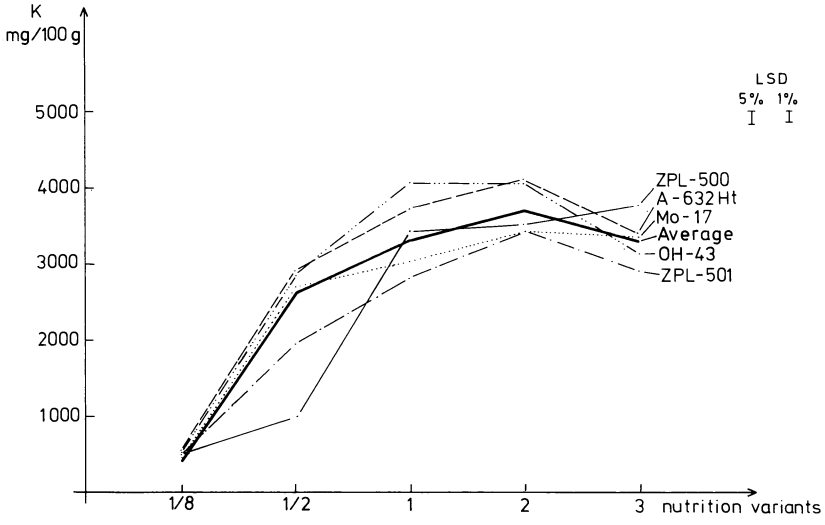

Fig. 8. Potassium concentration in roots of different inbred lines of maize.

nutrient elements in solution increased, in all inbreds examined. The line ZPL-501 had the lowest concentration in both aboveground part and root.

Discussion

Our results on dry matter mass of both aboveground part and root indicate considerable differences between maize genotypes dependent on the mineral nutrition. For example, the line ZPL-500 was distinguished by its average dry mass and A-632-Ht by a reduced value.

The experiments with 12 maize hybrids (Sarić and Kristić, 1978) show that difference in the organic matter synthesis approach 30 %. Kovačević (1980) analysing 10 inbred lines suggests that the synthesis of dry matter of both aboveground part and root may differ by 40 %.

Investigating 60 inbred lines Ramirez (1982) indicates that the production of dry matter per plant ranges from 133 to 331 mg, *i.e.* that certain

Table 1. Dry mass (mg/plant) and element concentration (mg/100 g) in different maize inbreds (average for all nutrition variants)

	ZPL-500	A-632-Ht	OH-43	ZPL-501	Mo-17	LSD	
						5%	1%
	Aboveground part						
Mass	512 a	361 c	486 a	499 a	424 b	28	37
N	3293 e	3408 d	3458 c	3526 b	3608 a	17	23
P	795 d	848 b	862 a	823 c	822 c	10	13
K	2469 ab	2486 ab	2532 a	2210 c	2440 b	68	91
	Root						
Mass	315 a	213 c	218 c	318 a	273 b	15	20
N	2492 a	2417 b	2468 a	2441 b	2472 ab	31	41
P	362 d	283 e	375 c	458 b	489 a	3	4
K	2791 b	2897 a	2794 b	2288 d	2616 c	52	69

inbreds are characterized by up to 2.5 times higher organic matter production.

Variations of microelements are found to be expressed to a greater extent than that of macroelements. Clark and Brown (1974) indicate that the variation percentages of Fe, Cu, Al, and Mn are 39, 49, 41, and 73 respectively.

Sarić and Kovačević (1978) report their results on content of nitrogen, phosphorus, potassium and magnesium in kernel, endosperm, and embryo of 10 maize inbreds and a number of their single hybrids. Total amount and distribution of the elements under examination were different and depended on genotype that indicates the genetic background of different ion accumulation in different genotypes.

By comparing the results on dry matter mass and element concentration (Table 1) it may be concluded on the basis of the averages by their weight but, their aboveground parts have not the highest content of the elements analyzed. Also, in their roots, no correlation is observed between root mass and element concentration.

Our results also show that the variation range of element concentrations depends upon the level of mineral nutrition. Therefore, at lower concentrations employed, smaller differences between the genotypes, with the exception of N concentration in aboveground parts, are obtained while the genetic specificity of mineral nutrition is evident particularly at optimum nutrition concentrations.

Investigation into the problem of the specificity of mineral nutrition in different plant genotypes still attempt to ascertain why a genotype takes up more ions and others less. In recent years, attention has been focused upon the enzymes conducting the processes of metabolism into various directions by the various activities. Investigation of the activities of primary enzymes of assimilation and metabolism of nitrogen with the same genotypes and the same nitrogen concentrations (Popović *et al.*, 1983) shows that the differences are found in the activities of their enzymes and their coenzymes. The author suggests that the activity of these enzymes might be used as a biochemical criterion in selecting both optimum nitrogen concentration and plant inbred line.

Data on the root and aboveground part morphology and anatomy, transpiration intensity, and element concentration of mineral nutrition (Merkulov, 1988) show that there are significant differences in concentration of mineral elements between certain genotypes, nutrition variants, vegetative organs and individual parts of the root system.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 33–37.
PLSO PN 126
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Accumulation and translocation of nitrogen in cultivars of winter wheat with different demands for nutrition

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Key words: nitrogen accumulation, nitrogen translocation, root growth, leaf area indices, cultivars of wheat

Abstract

Empirical differentiation of winter wheat cultivars into extensive and intensive types were confirmed by some physiological characters. Extensive cultivars were able to accumulate more protein in the vegetative tissues under reduced nitrogen fertilization than intensive types. Therefore they translocate more protein to the kernels. At the beginning of kernel filling they have more roots, which are able to absorb more nutrients. Correlations between the type of cultivar and the assimilation capacity could not be detected.

Introduction

Farmers, by empirical experience, separate cultivars of wheat into extensive and intensive types based on the amount of nitrogen fertilizer needed for high yield. Extensive cultivars are usually taller and have big loose ears with a high number of kernels and a high thousand kernel weight. Intensive cultivars are shorter, have compact ears with smaller kernels, a greater number of tillers and a higher number of ear bearing stalks.

The aim of the present study was to determine the extent of variation in the demand for nitrogen between these types and which physiological characters are responsible for this variation.

Material and methods

The study consisted of three experiments. Two of them were conducted 1987 in the field at an experimental station of the Bavarian Institute for Soil Fertility and Crop Production. In the field experiments the factors were: 1. Cultivars of winter wheat: Okapi and Ares (extensive) and Futur and Apollo (intensive). 2. Nitrogen fertilizer level: optimal for the site (140 kg N/ha) and reduced for 50% (70 kg N/ha).

Trial 1 for the determination of changes in dry matter content and protein had many replicates. The replicates were continually used for the weekly cuts. These were done on 3 areas of 0.25 m^2, beginning at tillering and finishing at maturity. Protein contents were analysed after Kjeldahl.

Trial 2 for the determination of yield and physiological characters had 4 replicates, 3 were used for the determination of yield, 1 for the determination of physiological characters. LAI's were measured in 3 replicates by leaf area meter (Hayashi Denkoh AAM). Yield components were determined on fixed areas of 0.25 m^2 in three replicates.

In Trial 3 the root growth of cultivars was tested in root container with two replicates. The containers were 0.9 m high, 0.2 m deep and 0.4 m broad. The containers were filled with soil from fields where the other trials were seeded. It was soil from the 2. horizont, compacted and fertilized equally. The planting density in the container was the same as in the field.

Results and discussion

Changes in dry matter and protein contents during growth

The weekly cuts of trial 1 were made to detect differences in growth and protein synthesis during

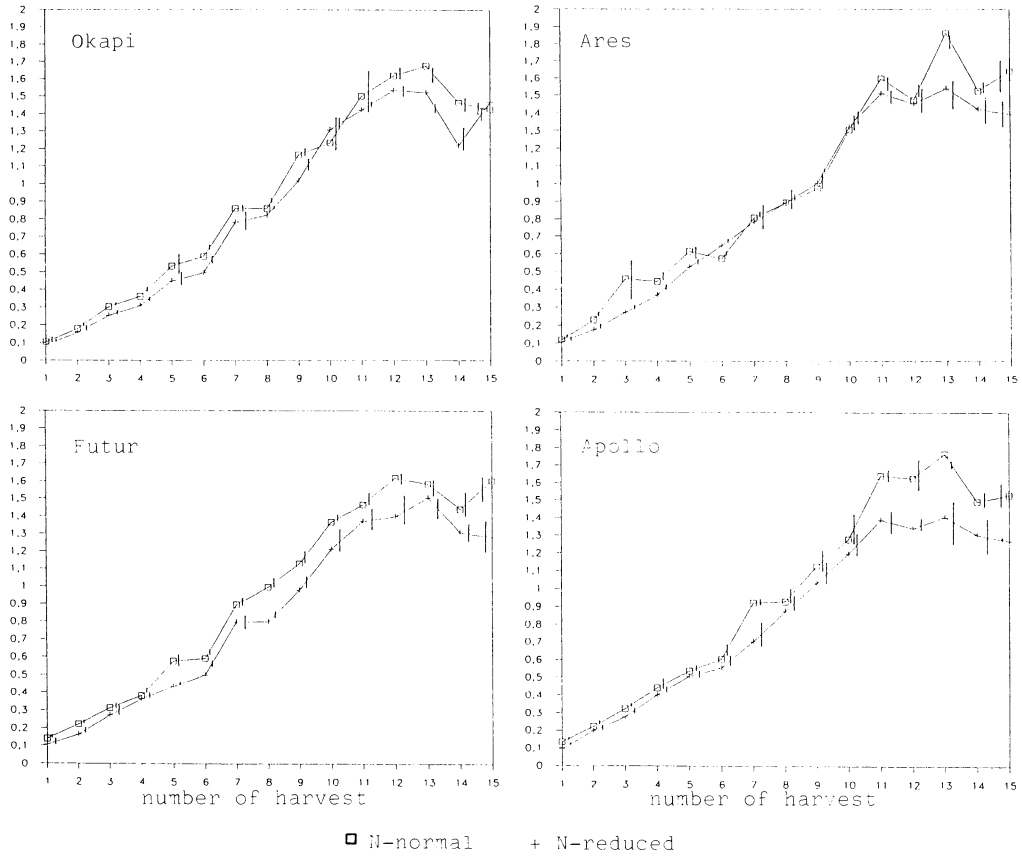

Fig. 1. Changes in dry matter content (kg/m^2 FM) of the whole plants.

the development of crops. Figures 1 and 2 show the accumulation of dry matter and protein of the 4 cultivars at 2 nitrogen fertilizer levels. The influence of nitrogen fertilizer on growth (Fig. 1) and protein content (Fig. 2) was smaller within the extensive cultivars Okapi and Ares than within the intensive cultivars Futur and Apollo. The enlargement of the differences in later growth stages is probably due to the higher loss of leaves in the intensive cultivars under reduced fertilization. The change in the slope before harvest 14 gives also an indication of a greater loss of leaves in the intensive cultivars (Fig. 2).

Translocation during kernel growth

The amount of protein translocation from leaves and stalks to the kernels under reduced fertilization is an essential physiological parameter for better nutrient efficiency.

The changes of protein yields in leaves and stalks of the cultivars differ distinctly as a result of nitrogen fertilization (Fig. 3). At harvest extensive cultivars had contents between 60 and 70 mg/g FM, while intensive cultivars had contents below 50 mg. Extensive cultivars seem to have a higher ability for the accumulation of protein in leaves and stalks irrespective of N-fertilizer inputs. The protein contents of all cultivars were equally low at maturity. Therefore the higher translocation rates under reduced fertilization from Okapi (49 g/m^2) and Ares (52 g/m^2) in comparison to Futur (36 g) and Apollo (35 g) result from the higher contents at the beginning of translocation and not from greater depletion of leaves and stalks.

The enrichment of protein in the kernels under reduced fertilization takes an analogous course

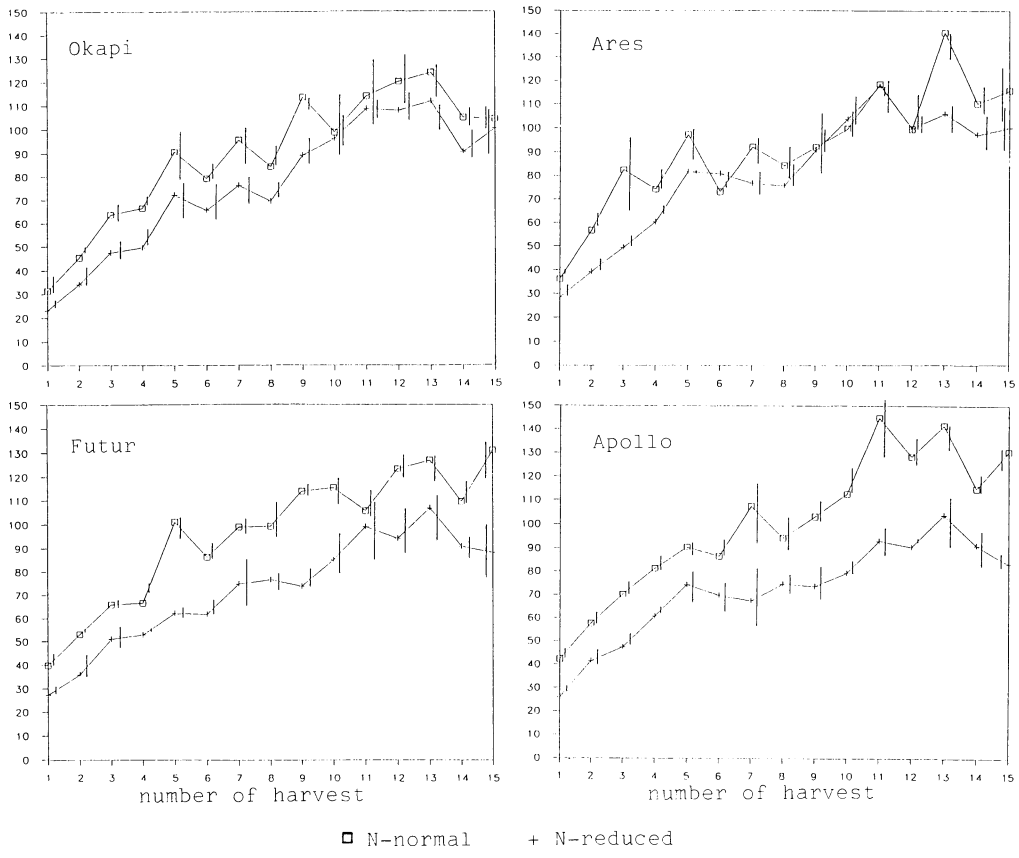

Fig. 2. Changes in protein content (g/m² FM) of the whole plants.

(Fig. 4). The contents of all cultivars were quite similar at the beginning of kernel filling. At maturity the extensive cultivars Okapi and Ares had contents of 53 and 48 g/m² and the intensive cultivars Futur and Apollo only of 38 and 39 g/m² at low N-levels. Under high fertilization the protein yields of kernels are different from those under reduced fertilization, and are correlated with the yield capacity of the cultivars under intensive cultivation methods.

Table 1. Root length of cultivars in containers at ear emergence (Stage 51)

Cultivar	Root length	Standard deviation
Okapi	399	14.3
Ares	330	6.6
Futur	265	10.8
Apollo	294	14.4

Differences in root growth of cultivars

The root growth of cultivars was examined to

Table 2. Leaf area indices

Cultivar	Fertilization (kg N)	LAI Stage		
		30	37	51
Okapi	140	4.2	5.9	6.3
	70	2.9	5.5	4.6
Ares	140	4.6	6.8	5.7
	70	3.6	4.0	4.0
Futur	140	4.3	6.5	4.8
	70	2.5	5.4	4.6
Apollo	140	5.8	7.8	5.6
	70	3.0	4.4	3.5

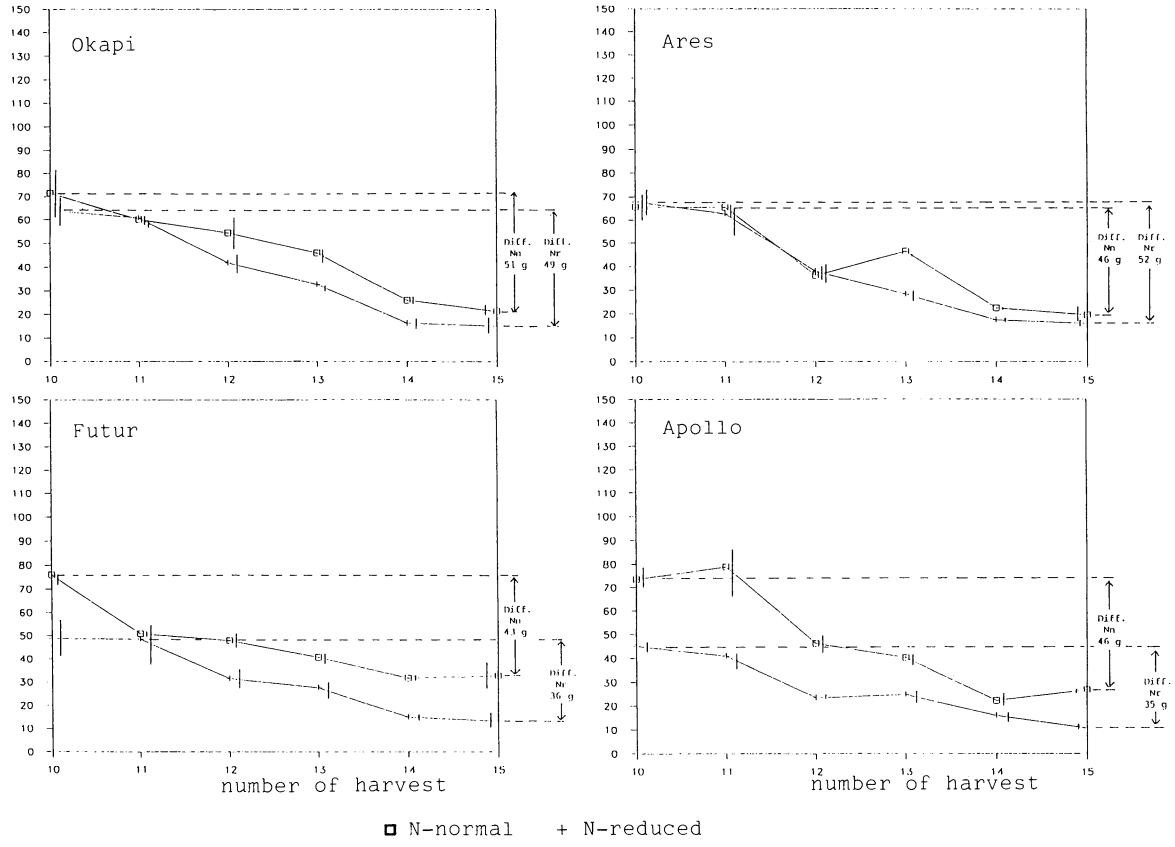

Fig. 3. Changes in protein content (g/m² FM) of leaves and stalks after flowering.

find an explanation for the differences in the accumulation of nitrogen. The extensive cultivars Opaki and Ares had more roots at ear emergence (Table 1), suggesting relationship between root length and N-accumulation in leaves and stalks.

Differences in the assimilation capacity of cultivars

Characteristics of assimilation capacity were examined to detect possible influences on protein synthesis in relation to N-supply.

The leaf area indices of cultivars diminished under reduced fertilization in all stages of growth (Table 2). Relationships between N-level and cultivar type could not be detected. No differences could be found in the length of kernel filling between the cultivars. The higher N-supply extended kernel filling period for two days within all cultivars. There is no indication that the accumulation of nitrogen or the translocation of protein was influenced by differences in energy supply.

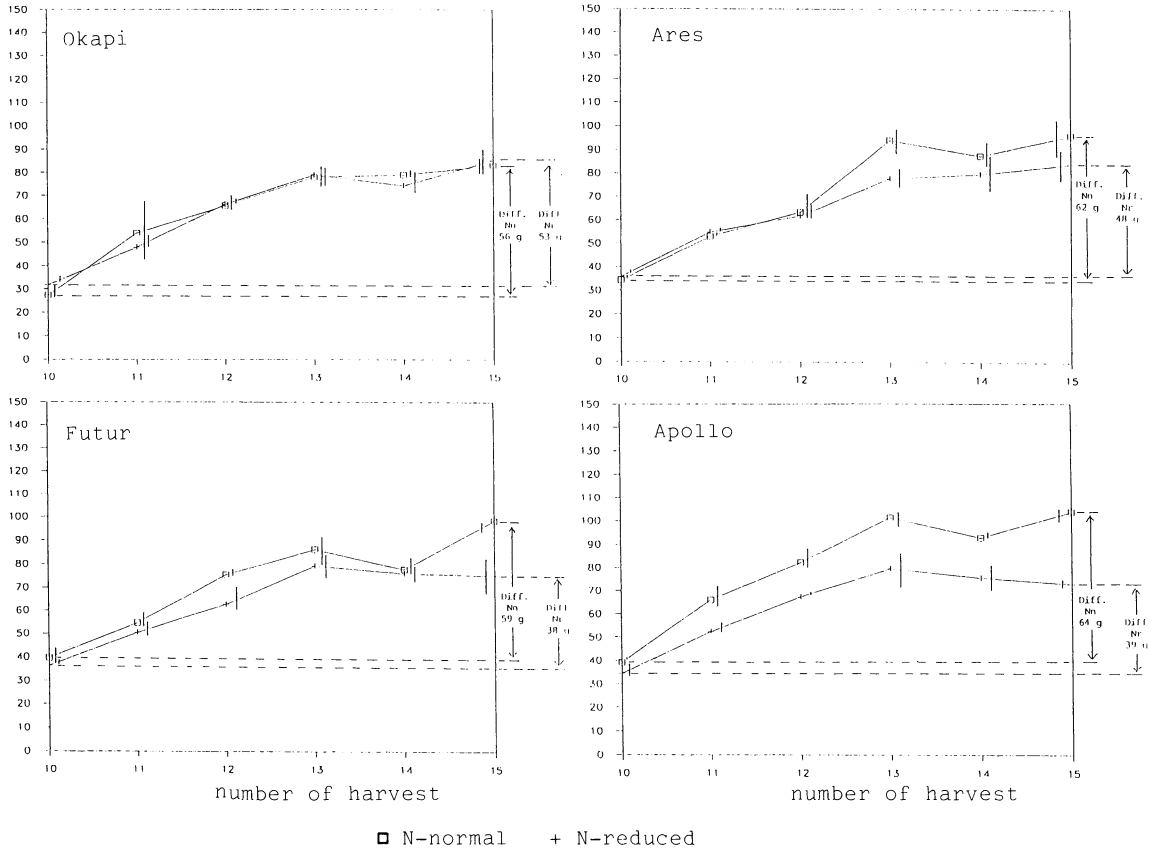

Fig. 4. Changes in protein content (g/m^2 FM) of ears after flowering.

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 39–43.
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PLSO PN 127

Uptake and partitioning of nitrogen in nitrogen-limited barley: Dependence of age and genotype

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Key words: growth, *Hordeum vulgare* L., solution culture, maturation, nitrate uptake, nitrogen partitioning, nitrogen limitation

Abstract

A nitrogen addition regime is described for growth of barley to maturation in N-limited solution culture. In 3 cultivars of barley, growth, nitrogen partitioning and nitrate uptake properties were studied every 14 days. The three cultivars differed from each other in partitioning of biomass and nitrogen between different plant parts, but the nitrate uptake properties were very similar. An ontogeny-linked decline in nitrate uptake capacity was observed, and could be explained by a decreased contribution of the root to total biomass production.

Introduction

When nitrogen is supplied in a single dressing in spring, soil nitrate level will drop during the growing season because of uptake by the crop, denitrification and leaching (Nielsen and Jensen, 1986). Cereal plants can, thus, be forced to support developing grains entirely through redistribution of nitrogen (Ebelhar *et al.*, 1987). Nitrogen can, however, be taken up from the soil also during grain-filling, and this nitrogen may be of importance for protein yield and grain-protein concentration (Cox *et al.*, 1985; Gregory *et al.*, 1981). Optimal use of fertilizer nitrogen requires better understanding of how the ability to absorb nitrogen changes during the life span of the plants. Cultivars having high uptake capacity or high affinity for nitrate during later stages of development may be important.

Experimental approaches to this problem are hampered by difficulties in monitoring root activities in the field. In solution culture, difficulties arise in controlling tillering and in obtaining full maturation of the plants under appropriate conditions of nitrate nutrition. This communication is a preliminary report on results obtained by applying the relative addition rate concept, according to Ingestad (1982), to solution culture of barley, grown to almost full maturation. The objective has been to study the capacity and affinity for nitrate transport, growth, development and nitrogen contents, as well as genotypic variation of these parameters during the life span of the plant.

Materials and methods

Culturing

Three cultivars of spring barley, (*Hordeum vulgare* L.) were used, namely Mette, Golf and var. *laevigatum*. Seeds were germinated for 3 days and then placed in 2 L beakers containing N-free nutrient medium (Ericsson *et al.*, 1982). Nitrogen additions started after 4 days (described below), and the medium was changed completely once a week. The plants were grown in a climate chamber with 16 h light (400 μmol $m^{-2}s^{-1}$, 18°C) and 8 h

darkness (12°C). The relative humidity was 70% and pH of the medium varied between 5.5 and 6.5. The plants were grown for 147 days. Uptake experiments with following harvests were performed every two weeks. At harvest, growth was monitored as root weight, leaf and stem weight, and ear weight. The nitrogen content of those plant parts were determined by an automated Dumas combustion method.

Theory of nitrogen application

The absorption of nitrogen by plants growing exponentially with adequate nitrogen supply and with completely stable internal nitrogen status can be described as

$$N_t = N_O \cdot e^{RGRt}$$

Where N_t and N_O denote the nitrogen contents of the culture at times t and zero, respectively and RGR denotes the relative growth rate. If, however, the flux of nitrogen to the culture is limited, but constant relative to the amount of nitrogen already bound in the culture, this expression can be recalculated to

$$N_t = N_O \cdot e^{RNt}$$

where RN is the relative rate of nitrogen increment in the culture. RN can be easily controlled by frequent additions of nitrogen to the culture to produce a relatve addition rate of nitrogen (RAN) which equals RN. RAN as the driving variable for growth has been used in numerous experiments primarily with perennials, with in general very good correlations between RAN and RGR (see Ingestad 1982, and Ingestad and Lund 1986, for reviews). This has also been found for population growth of Lemna (Ingemarsson *et al.*, 1984) and for growth of an annual species, Pisum, during a limited phase of vegetative growth (Oscarson and Larsson, 1986). Growth of an annual to maturation will, however, involve drastic changes in internal nitrogen status and nitrogen distribution, as well as in growth rate, but the overall flux of nitrogen to the culture can be controlled by RAN. The method has similarities to the "programmed nutrient addition technique" described by Cowie and Asher (1970, reviewed by Asher and Edwards 1983).

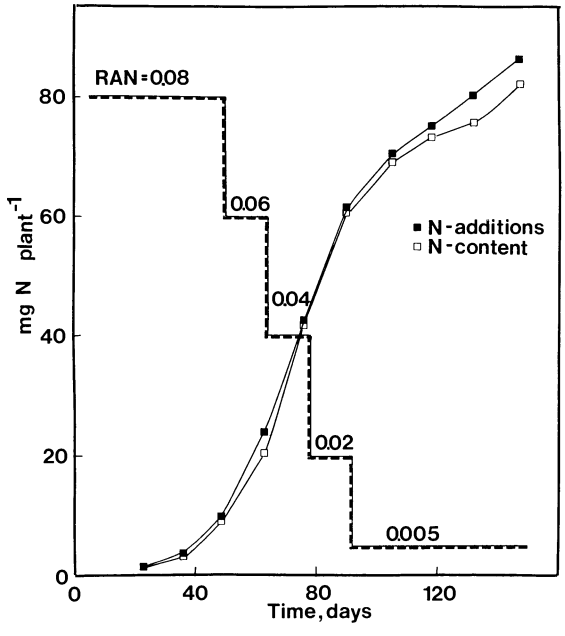

Fig. 1. Stepwise decreasing relative addition rate of nitrogen (RAN), cumulative N-additions to the culture, and actual N-content of the plants as mean values of cvs. Mette and Golf and var. *laevigatum* together.

Practice of nitrogen application

The nitrogen dose required for one day of growth at a chosen RAN was added as a single daily dose, together with all other elements of the basal nutrient medium. Two different strategies have been followed: 1) RAN was stepwise reduced during culturing from an initial rate of 0.08 day^{-1} during the early vegetative stage to 0.005 day^{-1} during grain-filling (Fig. 1). 2) RAN was kept constant at 0.0375 day^{-1} throughout the culturing period.

Nitrate uptake measurements

Nitrate uptake was measured as the initial rate of nitrate disappearance, by monitoring UV absorption continuously at 202 nm. Nitrate concentrations of 30, 70 and 150 μM were used in the uptake experiments.

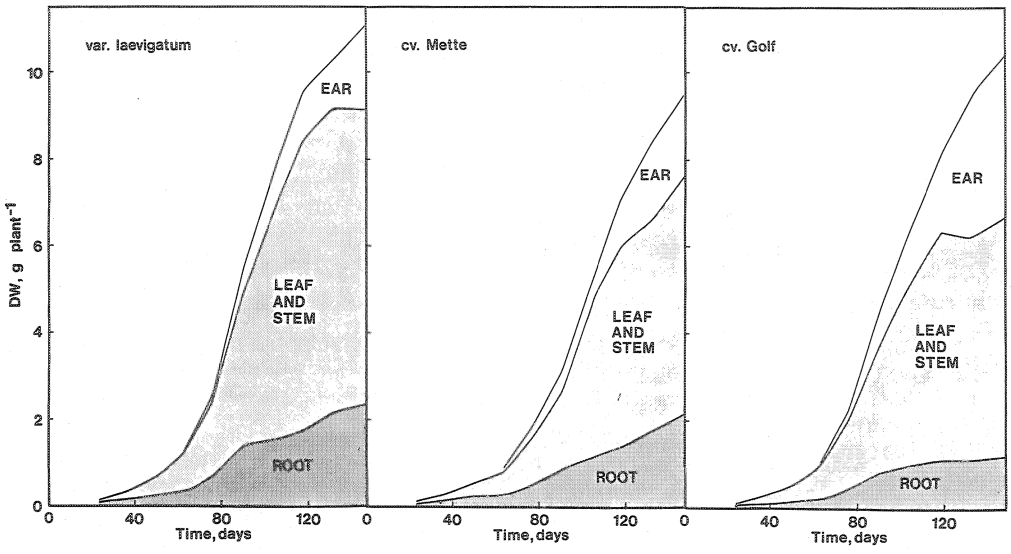

Fig. 2. Growth expressed as dry matter increase of root, leaf and stem and ear in cvs. Mette and Golf and var. *laevigatum. Each symbol* represents means of 2 parallel plant groups, each consisting of 3 plants.

Results and discussion

Growth and N partitioning

Figure 1 outlines the stepwise decreasing relative addition rate of nitrogen (RAN) as well as the cumulative amount of nitrogen added to, and recovered in the culture. On average, almost 95% of added nitrogen was recovered, which indicates that RAN controls nitrogen absorption of the cultures over the entire time span studied (thus, RN = RAN).

Figure 2 and 3 show partitioning of dry matter and nitrogen between the different plant parts of the 3 cultivars. Var. *laevigatum* had longest stems and also produced most total biomass (Fig. 2) but cv. Golf partitioned more biomass to the ears and did not produce as many leaves, stems and roots. Cv. Golf also developed a higher number of ears per plant (9) compared to the other cultivars (5 for cv. Mette and 6 for var. laevigatum). In nitrogen partitioning to the ears, cv. Golf was superior to the other cultivars (Fig. 3). In both cvs Mette and Golf the nitrogen content of the leaves and stems

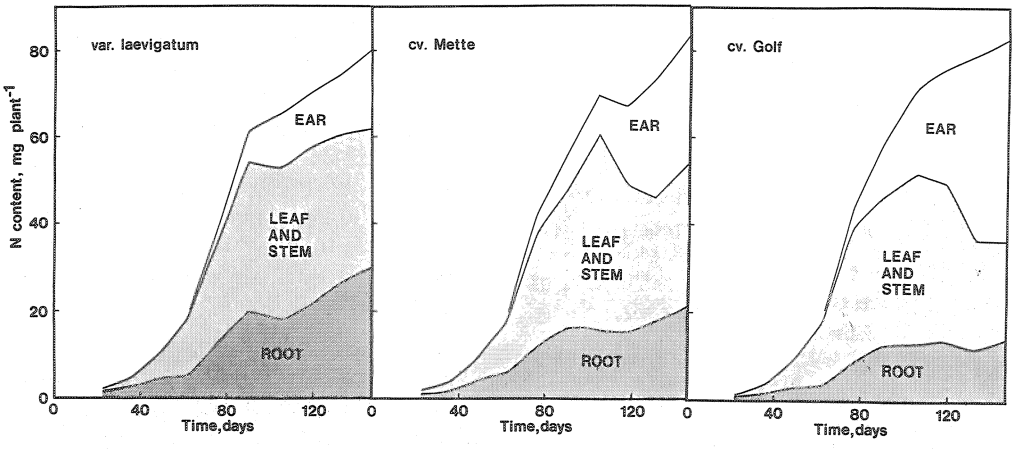

Fig. 3 . Nitrogen content of root, leaf and stem and ear in cvs. Mette means of 2 parallel plant groups, each consisting of 3 plants. 2 parallel plant groups, each consisting of 3 plants.

decreased after day 105 which means that net redistribution to the ears occurred during the last 6 weeks.

Nitrate uptake properties

The data on net nitrate uptake at 30, 70 and 150 μM were used to calculate apparent V_{max} values. To integrate V_{max} with the demand imposed on the uptake system in order to result in a specific RN, the V_{max} values given in Figure 4 have been transformed to relative units, *i.e.* g nitrogen taken up per g nitrogen present in the culture per day. The data in Figure 4 show that the 3 cultivars in the early vegetative stage have a nitrogen uptake capacity of well above 1 per day, which is more than 20-fold (RN = 0.08) or about 40-fold (RN = 0.0375) higher than the minimum capacity required to maintain RAN. Ageing is accompanied by a decline in uptake capacity which is somewhat less drastic for var. *laevigatum*. This decline is not a consequence of the stepwise reduction of RAN, as demonstrated by the very similar response in the treatment with constant RAN. The uptake capacity of the constant cultures eventually fell more than in cultures maintained at decreasing RAN, and there were indications that these cultures were in the end not N-limited.

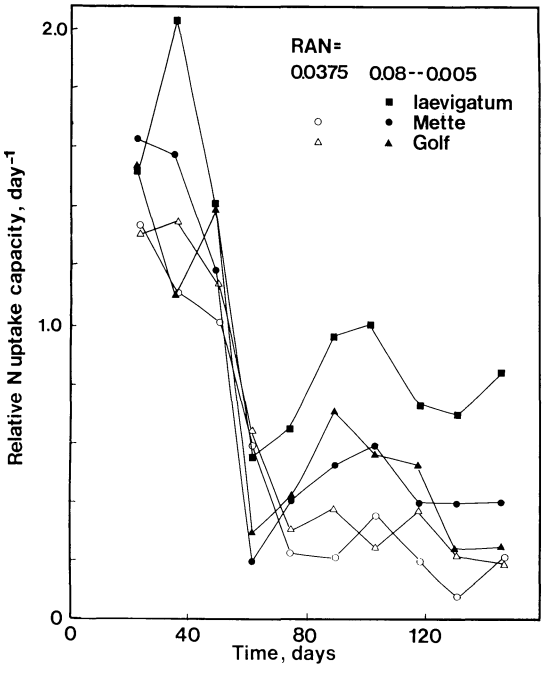

Fig. 4. Relative nitrogen uptake capacity for cvs. Mette and Golf and var. *laevigatum* cultured with stepwise decreasing RAN or for cvs. Mette and Golf with constant RAN. *Each symbol* represents means of 2 parallel plant groups, each consisting of 3 plants.

Figure 4 thus shows an ontogeny-linked decline in the capacity of the plant to absorb nitrate. To a major extent, this can be explained by a decrease in contribution of the root to total plant biomass which takes place from stem elongation onwards. Since this transition takes place at the same time for the 3 cultivars in both treatments, the decline in uptake capacity is simultaneous. A comparision of an early and a late developmental stage shows that the specific uptake capacity (*i.e.* expressed on a root weight basis) is relatively unaffected (Table 1). Also the apparent K_M is fairly unaffected although determinations of K_M on a very limited number of data points may be hazardous (see discussion by Lee and Drew, 1986).

The kinetic parameters derived from the uptake experiments can be used to simulate the steady state nitrate concentration at the root surface that would be required to sustain a specific RN, (here termed the balancing nitrate concentration). This can be regarded as an alternative definition of affinity, which integrates nitrate concentration at the root surface with nitrate flux to the root and the demand imposed on the root in order to maintain RN. Analysed in this way, the balancing concentration in the younger stages of plants cultured with stepwise reduced nitrogen availability is higher than in the older stages, mainly because RN decreases from 0.08 to 0.005 day^{-1} (Table 1).

Conclusions

The method utilized in this communication enables solution culture of cereals to near maturation while controlling both the nitrate nutrition and nitrate uptake properties. Genotypic variation was clear in N-allocation while there was less genotypic variation in uptake properties (Fig. 4, Table 1). Loss of nitrate uptake capacity with age is primarily associatated with decreased contribution of the root to total biomass. The implication of these ontogeny-linked changes in uptake properties on internal translocation of nitrogen and on grain-

Table 1. Kinetic parameters of nitrate uptake in 36 and 105-day-old barley plants of cvs. Mette and Golf and var. laevigatum. Each value represents means of 2 parallel plant groups each consisting of 3 plants

Cultivar	Age (days)	RAN (RN) (day^{-1})	V_{max}(root) ($\mu mol\, g^{-1}\, DW\, h^{-1}$)	K_M (μM)	Balancing NO_3^- concentration (μM)
Var. laevig.	36	0.08	132	71.4	2.7
	105	0.005	152	91.0	0.44
Cv. Mette	36	0.08	137	75.4	4.2
	105	0.005	107	66.6	0.45
Cv. Golf	36	0.08	110	40.3	3.2
	105	0.005	117	53.5	0.47

filling are currently being investigated in relation to genotypic variation.

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PLSO PN 128
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Dry weight production and nitrogen efficiency in cultivars of barley and rye

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Key words: barley, cereal, cultivar, dry weight, earliness, landrace, nitrogen efficiency, rye, water culture

Abstract

Plants of 54 rye and 53 spring barley cultivars were grown in water culture with either limiting (low N) or non-limiting (high N) nitrogen supply. Rye plants were harvested on day 36 (low N plants) or day 37 (high N plants). To compensate for cultivar differences in ear liness, barley plants were harvested in late vegetative phases.

Significant cultivar differences in dry weight production were obtained among high and low N plants of barley and rye. Dry weights of low N plants of both species were closely related to their fresh weights, while dry weights of high N plants were closely related to the dry weights of their tillers. Our conclusion is that there exist large differences between cultivars (and between individual plants) of barley and rye in ability to exploit low or high N supply. Since tillers can be removed without killing the plant, barley and rye plants that grow well with low or high N can be isolated with a non-destructive method.

Introduction

Whiteaker *et al.* (1976), Goodman (1977), and Ponnamperuma (1977) have described methods for isolation of genotypes that grow well with low or high supply of specific mineral nutrients. The water culture method developed by Whiteaker *et al.* (1976) is easy to handle since it includes no changes of media. It can be used to screen cultivars of many dicotyledons for ability to grow with low supply of several nutrients (Gabelman and Gerloff, 1982).

Screening of cultivars of cereals for ability to grow with low or high N supply is more complicated since the pattern of N supply strongly influences the growth of tillers (Perby and Jensén, 1987a and references therein), and thereby the N requirement. One of the basic criteria for a good selection method is that the phenotypes obtained are normal (Perby, 1986). To obtain a sound tiller development, water culture grown barley plants must be supplied with N regularly and in small amounts (Perby, unpublished results).

A complication with spring crops is that plants of different cultivars may be in different physiological phases even if they are grown under similar conditions. The problem is evident among spring barley cultivars where cultivar differences in earliness (time needed to reach maturity) and in mineral physiology are closely related (Perby and Jensén, 1984, 1987a, b). There is a great risk that a screening method will erratically favour either early or late cultivars. Since grainfilling induces drastic physiological changes (see results and references in Perby and Jensén, 1984; 1987a, b) which favours modern cultivars when properties of grainfilling plants are compared (Ekman, 1981), barley plants in the present experiments were harvested in late vegetative phases. It is simpler to obtain rye plants in a uniform physiological condition since most rye cultivars are winter crops. If not vernalized, they remain vegetative for a long period.

In this paper, we have used dry weight production of modern and old spring barley and non-vernalized winter rye cultivars to determine ability to grow well with low or high N supply. Differences in uptake, levels and distribution of N in the present plant material will be presented elsewhere.

Material and methods

The experiments were performed with 53 cultivars of barley (*Hordeum vulgare* L.) and 54 cultivars of rye (*Secale cereale* L.). About 20 cultivars of each species were modern. The rest were landraces and other old cultivars. Most of the cultivars are adapted to cultivation in Scandinavia.

Fifty seeds of each cultivar were germinated on moist filter paper in Petri dishes. The seeds were exposed to 10°C for one day and thereafter to 20°C for three days. For each cultivar, eight of the most healthy seedlings were carefully selected. They were inserted into plugs of plastic foam and mounted on a 4-mm thick disc of grey PVC. Plants were placed individually on plastic beakers containing 400 ml nutrient solution.

The three media used supplied nutrients at the same rate per plant and unit of time as the three media used by Perby and Jensén (1987a, b). All three media contained 0.6 m*M* $MgSO_4$ 0.6 m*M* KH_2PO_4, 0.3 m*M* Na_2HPO_4 and 0.12 m*M* Fe-EDTA. Micronutrients (except Fe) were given at 60 percent of the concentrations given by Hewitt and Smith (1975). The standard medium also contained 1.2 m*M* KNO_3 and 0.6 m*M* $Ca(NO_3)_2$. In the 10% and 2.5% nitrate media, 90 and 97.5 percent, respectively, of the nitrate was replaced by sulphate.

Plants were grown with two nitrate regimes. Plants grown with a non-limiting nitrate (high N; Perby and Jensén, 1987a) supply were supplied with standard medium from day 0, while those grown with limiting nitrate (low N) supply were supplied with 2.5% nitrate medium from day 0 to 16 and thereafter 10% medium. The media were replaced every fourth day.

The plants were placed in a greenhouse where the sunlight was complemented with light from mercury lamps (Philips 400 W, total irradiance 80–90 Wm^{-2}). The daylength was 16 h. In the barley experiment, the daylength was reduced to 15 h after day 42. The temperature was 18 ± 2°C and the relative humidity was about 65 percent.

Rye plants were harvested on day 36 (high N plants) or on day 37 (low N plants). Most barley plants were harvested when the first flag leaf was fully emerged. However, the main stems on some low N barley plants died before the flag leaf emerged. These plants were harvested when the top leaf on the main stem was at least half wilted. Since a few high N plants were visibly attacked by leaf mould, the remaining high N plants were harvested on day 57.

At harvest, each plant was divided into main stem, tillers (Briggs, 1978) and root. The fresh weights were determined in the barley experiment and for low N plants of seven rye cultivars. The following parameters were determined for each plant: length of the longest root, number of leaves on the main stem and the condition of individual leaves, other visible symptoms of N deficiency (mainly senescence of the main stem), number of vital small and large tillers and (for barley plants) day of harvest. Dry weights were determined after three days at 70°C.

Results and discussion

Barley

The differences in age at harvest (Fig. 1) illustrates the differences in time needed to reach the end of the vegetative period (Waldren and Flowerday, 1979). Compared with low N plants, high N plants of most cultivars needed a longer period to develop the first flag leaf (Table 1). Among the old cultivars, there are some extremes with shorter or longer vegetative periods than can be found among the modern cultivars.

As shown in Figures 1 and 2, the dry weights are related to the age at harvest; r was 0.93 and 0.41 for low N and high N barley plants, respectively. It is obvious that differences in ability to produce dry matter are larger among high N plants than among low N plants. For low N plants, the expected dry weight was 0.0128 × harvest day + 0.055 g ($n = 211$) and for high N plants the expected dry weight was 0.0371 × harvest day + 0.553 g ($n = 204$). The ratio

$$r/e = \frac{\text{real dry weight}}{\text{expected dry weight}} \quad (1)$$

gives an estimate of the relative performance of individual plants and cultivars. The cultivar differences in r/e ratios are shown in Table 1. For many cultivars the relative performance of high and low N plants differed significantly. The efficient cultivar Kajsa has been studied previously. Com-

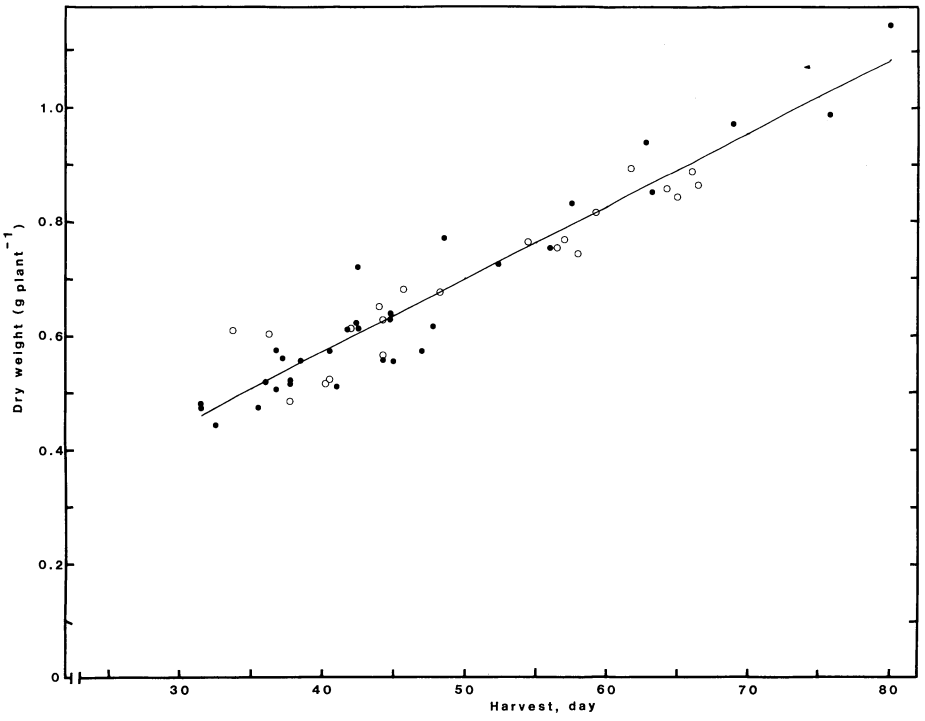

Fig. 1. Cultivar differences in mean age at harvest and mean dry weight of *low N barley plants*. Old cultivars are indicated by filled circles and modern cultivars by open circles. For harvest criteria, see Material and methods.

Table 1. Mean age at harvest and mean real/expected dry weight ratios (r/e, see Equation 1) for some barley cultivars grown with high or low N supply. The cultivars shown had the highest or the lowest mean r/e ratios in the high and low N experiments of the cultivars with short (< 39 days), intermediate (40–49 days) or long (> 52 days) vegetative periods in the low N experiment. Values are means for four plants. Data from Figures 1 and 2.

Cultivar	Low N plants		High N plants	
	Harvest day	r/e	Harvest day	r/e
Short vegetative period				
Kajsa	34	1.25	40	1.39
Gunilla	38	0.91	41	0.91
NGB 2064	32	1.03	29	0.66
Intermediate vegetative period				
NGB 421	42	1.21	57	1.01
Yrjar	48	1.00	50	1.47
NGB 315	47	0.87	48	0.88
NGB 437	48	0.92	57	0.63
Long vegetative period				
NGB 2068	63	1.09	57	0.80
NGB 4613	58	1.05	57	1.29
Roland	58	0.93	57	0.87
Kara	62	1.06	57	0.64

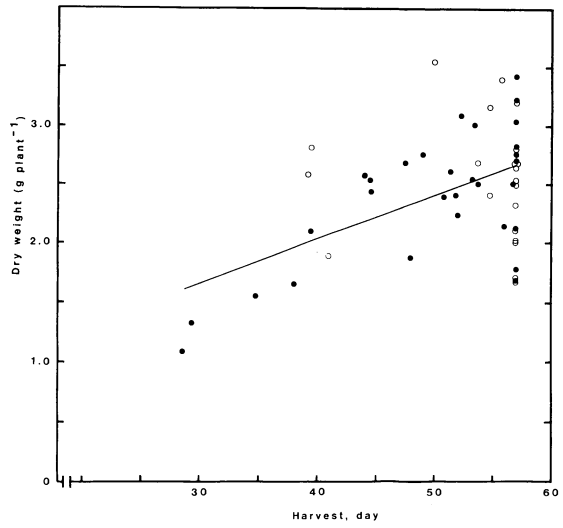

Fig. 2. Cultivar differences in mean age at harvest and mean dry weight of *high N barley plants*. Otherwise as for Fig. 1.

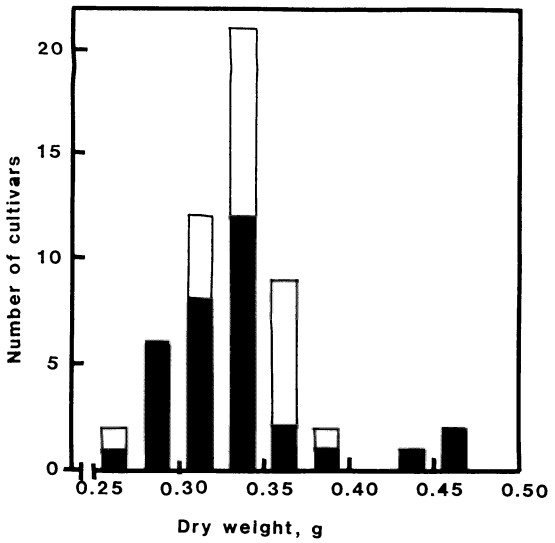

Fig. 3. Cultivar differences in mean dry weight of 36-day-old *low N rye plants*. The height of *each bar* gives the total number of cultivars with mean dry weight in the interval. Filled area indicates old cultivars and open area modern cultivars.

pared with a less efficient cultivar, its internal levels of N and rate of ^{15}N redistribution were lower (Perby and Jensén, 1987a, b).

Modern and old barley cultivars behaved as a uniform population in both experiments (Figures 1 and 2).

Rye

The mean dry weights of non-vernalized low and high N plants differ considerably between rye cultivars (Figures 3 and 4). There were no significant differences between the dry weights of modern and old cultivars. The relative differences in dry weight were larger among high N plants. The Finnish landrace NGB 59 produced least dry matter at the high N supply, only 46% of that of the best responder SMH 57. At the low N supply, the least efficient cultivar LPHl produced 54% of the dry matter of the most efficient, Midsommarråg ('Midsummer rye').

Non-destructive estimates of dry weight

The fresh weights of low N plants of both species were closely related to the dry weights (Table 2). For high N plants of both species, the differences in dry weights of tillers gave the best estimate of the

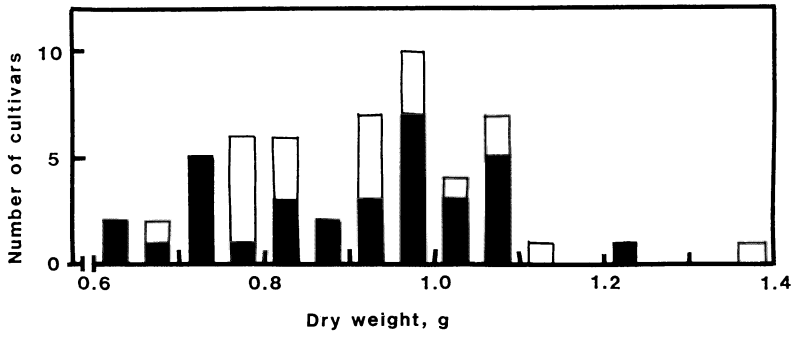

Fig. 4. Cultivar differences in mean dry weight of 37-day-old *high N rye plants*. Otherwise as for Fig. 3.

Table 2. Correlation coefficients (r) for the relations between some potential rapid screening parameters and the total dry weight of barley and rye plants. a = based on data from 28 plants of seven cultivars. nd = not determined

Parameter	Barley		Rye	
	High N	Low N	High N	Low N
Total fresh weight	0.211	0.948	nd	0.754[a]
Tiller dry weight	0.909	0.374	0.932	0.366
Number of tillers	0.490	0.188	0.517	0.062
Number of tillers with expanded leaves	0.474	0.262	0.517	−0.008
Number of dead leaves on the main stem	0.346	0.704	0.120	−0.079
Number of green leaves on the main stem	−0.157	−0.233	0.134	0.110
Number of leaves with no or small necrotic spots on the main stem	−0.290	−0.278	0.042	−0.123
Length of the longest root	−0.285	−0.183	−0.195	0.006
Number of plants	204	211	212	212

differences in dry weights of the plants. Since tillers can be removed without killing the plant, this method is non-destructive.

We performed a check to find whether differences in some easily determined parameters could be used as estimates of differences in dry weight. They were generally not reliable (Table 2).

Conclusions

For both barley and rye, cultivar differences in dry weight were largest among high N plants. Nevertheless, there exist large differences among cultivars, and between individual plants, of barley and rye in ability to grow with a limiting or a non-limiting N supply (Figures 1–4, Table 1) as has been shown for other species (Gabelman and Gerloff 1982; Goodman, 1977; Ponnamperuma, 1977). Gabelman and Gerloff (1982) have studied the heritability of differences in efficiency in some dicotyledonous species. Visual parameters, as used by Ponnamperuma (1977) and Gabelman and Gerloff (1982), cannot be used to estimate differences in dry weight among barley and rye plants.

There were no significant differences between dry weights of modern and old cultivars in ability to grow well with high or low N supply. Similarly, Cacco *et al.* (1983) obtained no significant differences in fresh weight of corn hybrids of different ages.

Acknowledgements

The work was supported by a grant from Erna and Victor Hasselblad's Foundation. Seeds were kindly provided by Svalöf A B, Svalöv, W Weibull A B, Landskrona and the Nordic Gene Bank, Alnarp, Sweden.

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PLSO PN 129

Nitrate reductase in sugar beet genotypes supplied with different nitrate levels

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Key words: genotypes dry matter, nitrate, nitrate reductase, sugar beet, total nitrogen

Abstract

The effect of different nitrate concentrations (1, 5 and 15 m*M*) was studied on nitrate reductase activity (NRA), NO_3 and total nitrogen content in different sugar beet genotypes grown in water cultures. Increasing NO_3 concentrations in the nutrient solution significantly increased the dry weight of the above-ground plant part and decreased the dry weight of roots. The nitrate and total nitrogen content as well as initial and total NRA significantly increased in shoots and roots with the increase of nitrate concentration in the nutrient medium. The distribution of NRA in plants was also affected by the levels of NO_3. At 1 m*M* and 15 m*M* 90% and 70% respectively was reduced in the leaves. The genotypes reacted differently to the increase of NO_3 concentration in nutrient solution. The genotypes with high amounts of nitrates and total N were found to have high initial and total activity of NR.

Introduction

Perennial plants usually take up nitrogen in the form of nitrates and the absorbed nitrates must be reduced to ammonium before incorporation in organic compounds. The reduction of nitrates is facilitated by the nitrate reductase (NR) (E.C.1.6.6.1). NR belongs to the group of enzymes, which are induced by substrate, thus the presence of nitrates in the medium significantly increases its activity (Beevers and Hageman, 1969; Meeker *et al.*, 1974; Yau and Thurling, 1987).

The distribution of the capacities for reduction of nitrates in particular organs of different plant species is unequal. In sugar beet plants, nitrate reduction is mostly performed in the above-ground organs. Nitrate influx in leaves significantly affects the level of NR activity (NRA) in them. However, many authors observed that the nitrate absorbed by plant organs does not necessarily undergo reduction. *i.e.*, nitrate absorbed by the root may be transported to the vacuole and accumulate in a reserve pool or be translocated into the cytoplasm and included in metabolitic pools. The activity of some enzymes is regulated by nitrates, present in the metabolitically active pool, *i.e.* nitrate which is directly transported to the site of reduction after uptake (Chanatarotwong *et al.*, 1976; Grant *et al.*, 1970).

The level of NRA as an indicator of the resistance of hibrid maize lines and/or wheat varieties to environmental factors, *e.g.* temperature, drought *etc.*, showed positive correlation in most cases (Alofe *et al.*, 1973; Amos and Scholl, 1977; Nair and Abrol, 1977).

Taking this in consideration, as well as the specific significance of nitrogen for sugar beet yield and quality, we studied NRA in particular sugar beet genotypes with their supplied with NO_3. We should also take in consideration that biogenesis of NR enzymes is controlled by the genes of both nucleus and cytoplasm (Edwards, 1974; Hageman and Flesher, 1960) and that NRA as a biochemical criterion for the assesment of wheat and maize supply with nitrogen is already successfully used (Croy and Hageman, 1970; Mugwira *et al.*, 1980).

Materials and methods

The experiments were performed with young sugar beet plants (*Beta vulgaris* L.). Seven genotypes were included in the experiments: KW-Lena (I), KW-Maja (II), Synemo (III), Gemo-39 (IV), Al-Cermona (V), NS-Hy-21 (VI), Maribo (VII). Of these genotypes KW-Lena and Synemo belong to the yielding type, KW-Maya, NS-Hy-21 and Maribo are classed as sugar type and Gemo-39 and Al-Cermona as normal type.

After they were germinated in Vermiculite, they were grown thirty days in the greenhouse in nutrient solution with three levels of NO_3 and these were: 1.0, 5.0, 15.0 m*M* NO_3. Iron was added to nutrient medium in the form of: Fe^{III}-EDTA.

The NRA was determined in 20-day old leaves and root segments by an *in vivo* method. The initial activity was determined in phosphate buffer pH 7.4, and total in the same buffer which contained 0.25 *M* KNO_3, by the application of Sulphanilamide (1.0 g in 100 ml in 1.5 *M* HCl) and N-(l Naphtyl)-ethylendiamine dihydrochloride (0.1 g in 100 ml of H_2O). The nitrate content in roots and leaves was determined by spectrophotometric assay with phenoldisulphic acid, and the content of total nitrogen by the method of Kjeldahl.

Results

Dry weight

Dry weight of the above-ground plant part and root of the genotypes depended significantly on their genetic specificity and nitrate supply (Table 1). The lowest dry matter of the above-ground plant part was found with the lowest concentration of NO_3. With increase of NO_3 concentration the dry matter of the above-ground part increased significantly, while that of the root decreased. Genetic variability of the dry matter of the genotypes is significantly expressed, especially in the case of its accumulation in the above-ground part. The greatest effect of NO_3 concentration was shown in the Synemo genotype of the yielding type, then in the normal genotypes and the lowest effect was observed in the representatives of the sugary type.

Nitrate reductase activity

The total and initial NRA of the above-ground plant parts depended on the genotype and concentration of NO_3 in the nutrient solution (Fig. 1). The results undoubtedly indicate that sugar beet

Table 1. The effect of different concentrations of NO_3-N in the nutrient solution on the dry weight (mg plant^{-1}) in different sugar beet genotypes

m*M* NO_3		Genotypes (B)						
(A)		I	II	III	IV	V	VI	VII
Above-ground part								
1.0		557	557	425	557	658	668	652
5.0		632	727	605	669	810	751	719
15.0		600	753	691	767	774	739	747
				A	B	AB		
LSD	5%			71.8	109.7	189.9		
	1%			95.5	145.8	252.6		
Roots								
1.0		203	241	252	235	237	271	254
5.0		225	263	182	205	270	215	201
15.0		174	228	174	172	200	165	169
				A	B	AB		
LSD	5%			12.7	19.2	33.4		
	1%			16.8	25.7	44.5		

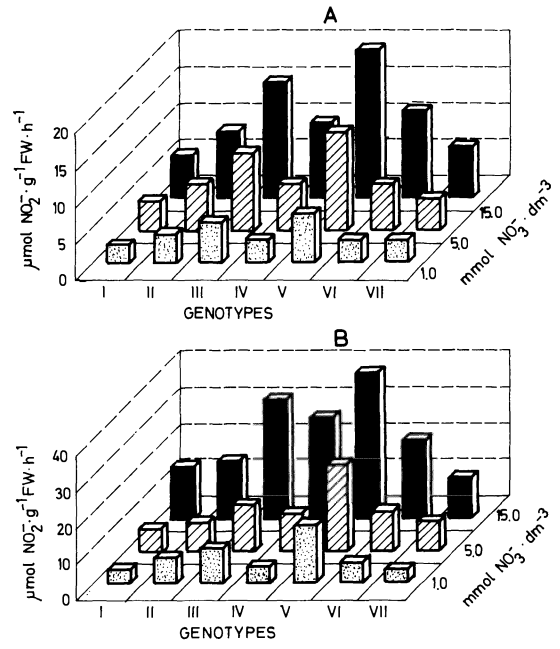

Fig. 1. The effect of different concentrations of NO_3-N on the nitrate reductase activity in the above-ground part at different sugar beet genotypes (**A** = initial NRA; **B** = total NRA).

leaves have a high capacity for NO_3 reduction. NRA varied among the studied genotypes. The variability was especially marked in high-yielding and normal genotypes in conditions of optimum or excessive NO_3 supply. However, in these conditions, the above genotypes showed higher capacity in NO_3 reduction than the sugar genotypes.

The initial and total NRA in root of the genotypes is also directly related to NO_3 concentration (Fig. 2). The results also indicate that the capacity for NO_3 reduction in sugar beet roots is small. NRA in roots of the genotypes was approximately the same, except in Al-Cermone, where it was significantly higher. The ratio of NRA of roots and above-ground plant parts significantly depended on the plant supply of nitrates.

The content of NO_3-N

The content of nitrates in the sugar beet genotypes is directly related to nitrate concentration (Fig. 3). The high content of NO_3 in the roots of sugar beet plants shows a significant degree of accumulation. The content of NO_3 among the

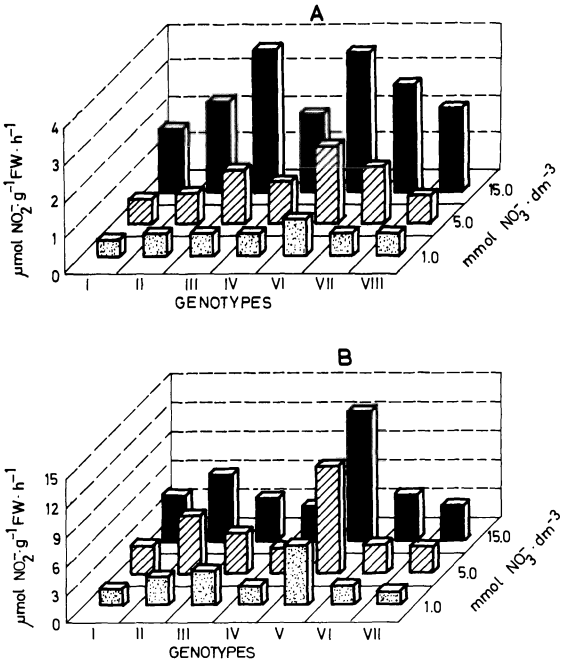

Fig. 2. The effect of different concentrations of NO_3-N on the nitrate reductase activity in the root at different sugar beet genotypes (**A** = initial NRA; **B** = total NRA).

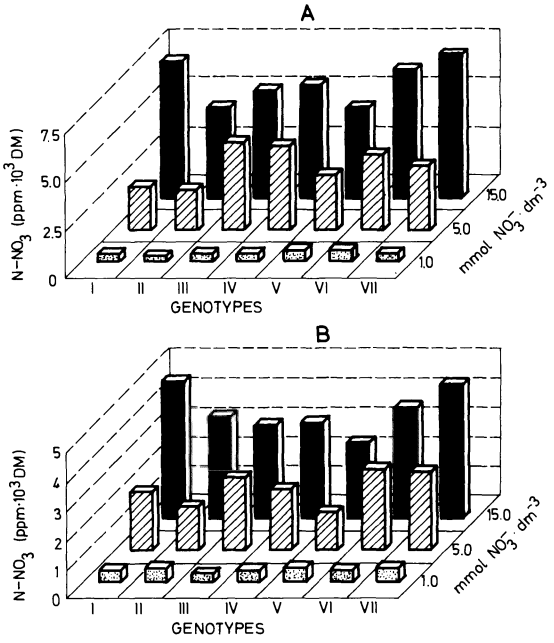

Fig. 3. The effect of different concentrations of NO_3-N on the content of nitrates in different sugar beet genotypes **A** = above-ground part; **B** = root).

Table 2. The effect of different concentrations of NO_3-N in the nutrient solution on the dry weight (mg · plant^{-1}) in different sugar beet genotypes

m*M* NO_3 (A)		Genotypes (B) I	II	III	IV	V	VI	VII
Above-ground part								
1.0		2.30	3.17	2.05	1.99	2.17	2.40	2.28
5.0		2.86	2.67	2.81	3.03	2.57	3.22	3.13
15.0		3.28	3.23	3.32	3.24	3.94	3.79	3.66
			A	B	AB			
LSD	5%		0.22	0.24	0.37			
	1%		0.29	0.32	0.48			
Roots								
1.0		1.82	1.61	1.56	1.62	1.78	1.78	1.67
5.0		2.41	2.17	2.06	2.48	1.95	2.43	2.08
15.0		2.64	2.39	2.57	2.59	3.00	3.04	2.98
			A	B	AB			
LSD	5%		0.18	0.21	0.30			
	1%		0.23	0.27	0.38			

genotypes significantly varied at all levels of NO_3 supply. The data analysis obtained in relation to NRA (Figures 1 and 2) and NO_3 content indicates that the genotypes with high NRA have a low NO_3 content and *vice versa*.

The content of total N in the genotypes and the concentration of NO_3 in the nutrient solution are also in positive correlation (Table 2). However, the obtained results at the same time indicate that the variability of the content of total N related to the investigated factors is, unlike NO_3-N, poorly expressed.

Discussion

On the basis of the results obtained in these experiments, as well as other data from the literature, we may conclude that about 85% of NO_3 uptake is translocated to the above-ground plant organs, *i.e.* that in sugar beet plants NO_3 reduction is mostly performed in the above-ground organs. However, we should take in consideration that the accumulation of nitrates in particular organs depends not only on the site of their reduction, but also on the supply of NO_3. The significant accumulation of NO_3 in the root of the sugar beet genotypes growing in conditions of the excessive nitrate supply obviously proves the previous opinion. The accumulation of NO_3 in particular organs shows that their capacity for nitrate reduction is low, or that the their uptake rate is more intensive than the reduction process. Thus, in organs of plant species and/or genotypes (Figures 1 and 2) which possess a high capacity for NO_3 reduction, NRA and NO_3 content in the medium are directly related, while in the species with low capacity for NO_3 reduction, the positive correlation is limited (Friedrich *et al.*, 1977). Taking this in consideration, we may conclude that NRA may be used as a criterion of the assessment of plant supply of nitrogen in plant species which are characterized by a high capacity for NO_3 reduction. This is also proved by the fact that many authors have observed better correlation among NRA and nitrogen concentration in the medium, studying the effect of lower nitrogen doses on enzymic activity (Mugwira *et al.*, 1980; Yau and Thurling, 1987). The use of NR enzymes as biochemical indicators for the results related to the content of NO_3 is justified (Fig. 3). However, it should be taken into consideration that the range of NRA depends on the ability of a genotype to adapt its total metabolism to the existing ecological conditions. The investigations of Croy and Hageman (1970) showed that NRA significantly depends not only of genotypic origin of genotype, but also of other ecological factors, geographic origin of genotype *etc.* If con-

sideration is given to the fact that the analyses were performed in semi controlled conditions and that the genotypes differ not only genetically, but also in origin, we may conclude that NRA of particular genotypes significantly depended on the realization of its genetic potential in the existing conditions of the investigations.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 57–63.
PLSO PN 130
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Influence of the nitrogen level on root growth and morphology of two potato varieties differing in nitrogen acquisition

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Key words: nitrogen acquisition, potato, root morphology, *Solanum tuberosum*

Abstract

Two potato (*Solanum tuberosum* L.) cultivars ('Astrid' and 'Bodenkraft') differing in their nitrogen acquisition from the soil (Hunnius, 1981) were used in nutrient solutions to study the effect of increasing concentrations of nitrate (0.05; 0.5; 5.0 mol m^{-3}) particularly on root growth and morphology. In each variety increasing nitrogen concentrations stimulated shoot growth more than root growth. At all nitrate concentrations, the variety with higher nitrogen acquisition ('Astrid') had a significantly larger root system. The larger root system of 'Astrid' compared to 'Bodenkraft' was particularly evident when surface area and total length of the roots, instead of root dry weight were used as parameters. The results stress the importance of root length and surface area for nitrogen acquisition from soils.

Introduction

Genotypical differences in nitrate efficiency of crop plants are well documented, for example, in maize (Muruli and Paulsen, 1981); oat (Brinkman and Rho, 1984; Cregan and Berkum, 1984) and wheat (Woodend *et al.*, 1985). For potato, however, in this respect relatively little information is available (Kleinkopf *et al.*, 1981). On the basis of both field and pot experiments with different levels of nitrogen fertilizer supply, Hunnius (1981) classified the potato cultivar 'Astrid' as nitrogen efficient, and the variety 'Bodenkraft' as nitrogen inefficient. Such genotypical differences may be due to both utilization efficiency (Moll *et al.*, 1982), *i.e.* dry matter production per g nitrogen taken up, and nitrogen acquisition by the roots (Perby and Jensen, 1983). Especially where nitrogen availability is low, nitrogen acquisition appears to be by far the more important factor (Anderson *et al.*, 1984). High nitrogen acquisition may be due to both the size of the root system (Chloupek, 1976) and root activity (Mugwira *et al.*, 1980). The part played by the latter factor, however, appears to be relatively minor under field conditions. Thus it may be concluded that under these conditions the size of the root system is the most important factor determining nitrogen acquisition.

Although the effect of increasing nitrogen supply on root growth has attracted broad interest (Bosemark, 1954; Drew *et al.*, 1973; Geisler, 1986), genotypes with different nitrogen efficiency were included in only a few studies (Hackett, 1968; Geisler and Krützfeld, 1983).

The aim of the present study was to compare the root morphology of the two potato varieties in general, and its response to different nitrogen levels in particular.

Material and methods

Cuttings of the two cultivars 'Astrid' and 'Bodenkraft' were cultivated in an aerated nutrient solution (5 l pots) in a growth cabinet. The nutrient solution was renewed twice a week. The compo-

sition of the standard nutrient solution used is based on Krauss (1978). Macronutrients: (mol m^{-3}): N, 5.0; P, 0.5; K, 2.0; Ca, 1.0; Mg, 0.4 and S, 1.5 as $Ca(NO_3)_2$; $Mg(NO_3)_2$; K_2SO_4 and KH_2PO_4. Micronutrients: (mmol m^{-3}): Fe, 13.8; Mn, 3.6; Cu, 1.6; Zn, 1.5; B, 55.5; Mo, 1.0 as FeEDTA; $MnSO_4$; $CuSO_4$; $ZnSO_4$; H_3BO_3 and $(NH_4)_6Mo_7O_{24}$. Three NO_3^- levels were used (5; 0.5; 0.05 mol m^{-3}) and NO_3^- was balanced by SO_4^{2-}. Experiments were carried out at 20°C/15°C day and night temperature, 14 h daylength, a relative humidity of 80% and a radiation energy at canopy level of 250 W m^{-2} (PAR).

Root surface area was determined by the ink method (Sattelmacher *et al.*, 1983), root volume and root morphology were studied according to Sattelmacher (1987). All data presented are the mean of four replications.

Results

In both cultivars shoot and root dry weight increases with increasing nitrate supply. Root fraction, however, declines *i.e.* the roots becoming relatively smaller (Fig. 1). This effect on root dry weight is especially pronounced in the variety 'Astrid', which has a higher root dry weight than 'Bodenkraft' at all nitrate concentrations. The genotypical differences in respect to root growth are shown in a representative example in Fig. 2.

In spite of differences in the size of the root system, the concentrations of the mineral elements in the dry matter of the two cultivars do not significantly differ (Table 1). This is due to a higher uptake rate per unit root surface area in 'Bodenkraft' compared to that of 'Astrid'. Despite the relatively small increase in root dry weight with the increasing nitrate supply (Fig. 1), the root surface area is considerably extended, particularly in 'Astrid' (Fig. 3). In both cultivars this extension is mainly the result of lateral root formation (Fig. 4). At high nitrate concentrations 'Astrid' forms longer laterals than 'Bodenkraft', which explains

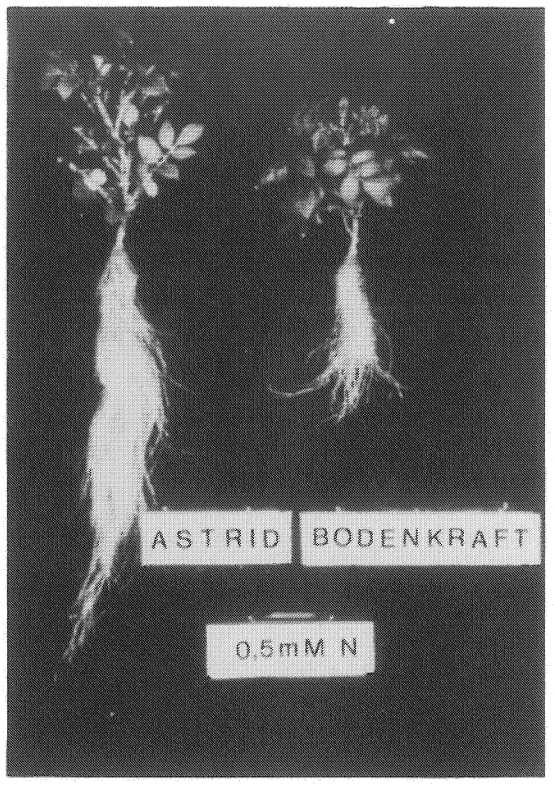

Fig. 2. The varieties 'Astrid' and 'Bodenkraft' grown at NO_3^- concentration of 0.5 mol m^{-3} for three weeks.

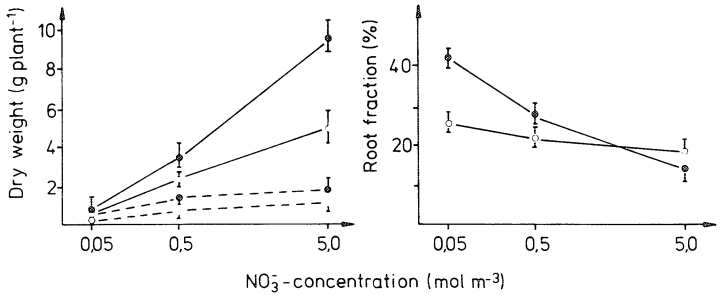

Fig. 1. Influence of the NO_3^- concentration in the nutrient solution on shoot (*solid line*) and root (*dotted line*) dry weight and the root fraction, *i.e.* root expressed as per cent of total plant dry weight, of the two potato varieties 'Astrid' (*solid symbols*) and 'Bodenkraft' (*open symbols*) three weeks after onset of the N treatment.

Table 1. Influence of NO_3^- supply on shoot and root concentration and on total uptake of mineral nutrients and on uptake rate per unit root surface area in the varieties "Astrid" and "Bodenkraft"

N-level (mol m^{-3})	Mineral nutrient concentration (mg g^{-1} dry matter)									
	Shoot					Root				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
"Astrid"										
0.05	27.5	5.8	34.0	8.8	13.5	30.0	13.3	29.5	2.7	7.8
0.5	41.8	6.3	46.5	10.3	13.5	36.0	16.5	41.3	2.8	7.3
5.0	63.5	7.4	64.3	22.5	8.8	51.3	10.8	41.0	5.9	7.5
LSD 5%	3.9	0.6	4.3	1.5	1.9	3.2	1.9	2.1	0.8	1.0
"Bodenkraft"										
0.05	33.3	6.7	38.8	9.5	12.5	34.8	8.5	27.5	4.3	9.3
0.5	52.3	7.3	45.5	11.3	13.8	40.5	11.0	34.8	3.0	11.5
5.0	64.5	7.4	52.5	24.5	9.3	48.3	9.3	32.3	5.0	12.3
LSD 5%	4.1	0.7	4.9	1.6	1.7	2.9	2.2	1.8	0.9	1.4
	Nutrient in the plant (mg plant^{-1})					Uptake rate (mg dm^{-2} d^{-1}				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
"Astrid"										
0.05	27	9	32	6	12	0.4	0.1	0.5	0.1	0.2
0.5	187	44	212	38	55	0.6	0.1	07	0.1	0.2
5.0	704	75	608	225	95	1.4	0.1	1.4	0.4	0.2
LSD 5%	140	26	150	39	15	0.7	0.1	0.8	0.2	0.1
"Bodenkraft"										
0.05	19	4	22	5	8	0.9	0.2	1.0	0.2	0.4
0.5	142	25	130	27	41	1.2	0.2	1.1	0.2	0.3
5.0	377	46	299	127	57	2.2	0.3	1.8	0.7	0.3
LSD 5%	133	24	128	28	20	0.7	0.1	0.8	0.2	0.1

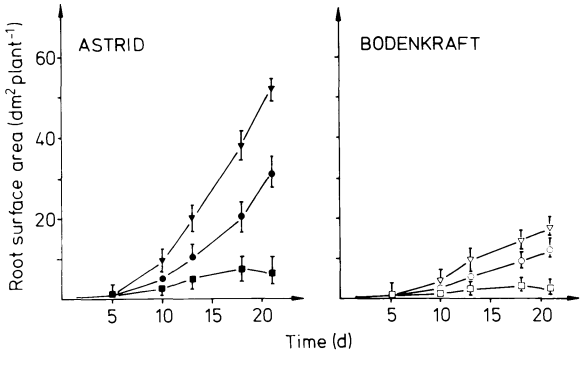

Fig. 3. Influence of NO_3^- concentration of the nutrient solution on root surface area increment of the varieties 'Astrid' (*solid symbols*) and 'Bodenkraft' (*open symbols*). (■ = 0.05; ● = 0.5, ▼ = 5.0 mol m^{-3}).

the differences between the two varieties in respect to the root surface area (Fig. 3). At the lowest nitrate concentration a cluster-like lateral root formation occurs in both varieties (Fig. 4). This pattern in lateral root formation is obviously the result of periodical changes in the nitrogen supply (depletion/resupply). Immediately after the renewal of the nutrient solution, abundant lateral root formation occurred, but after exhaustion of nitrate in the nutrient solution lateral root formation almost ceased.

The effect of increasing nitrate concentrations on root dry weight, root volume, root surface area and root length (Table 2) reveals that root dry weight is least affected. This reflects the enhanced lateral root

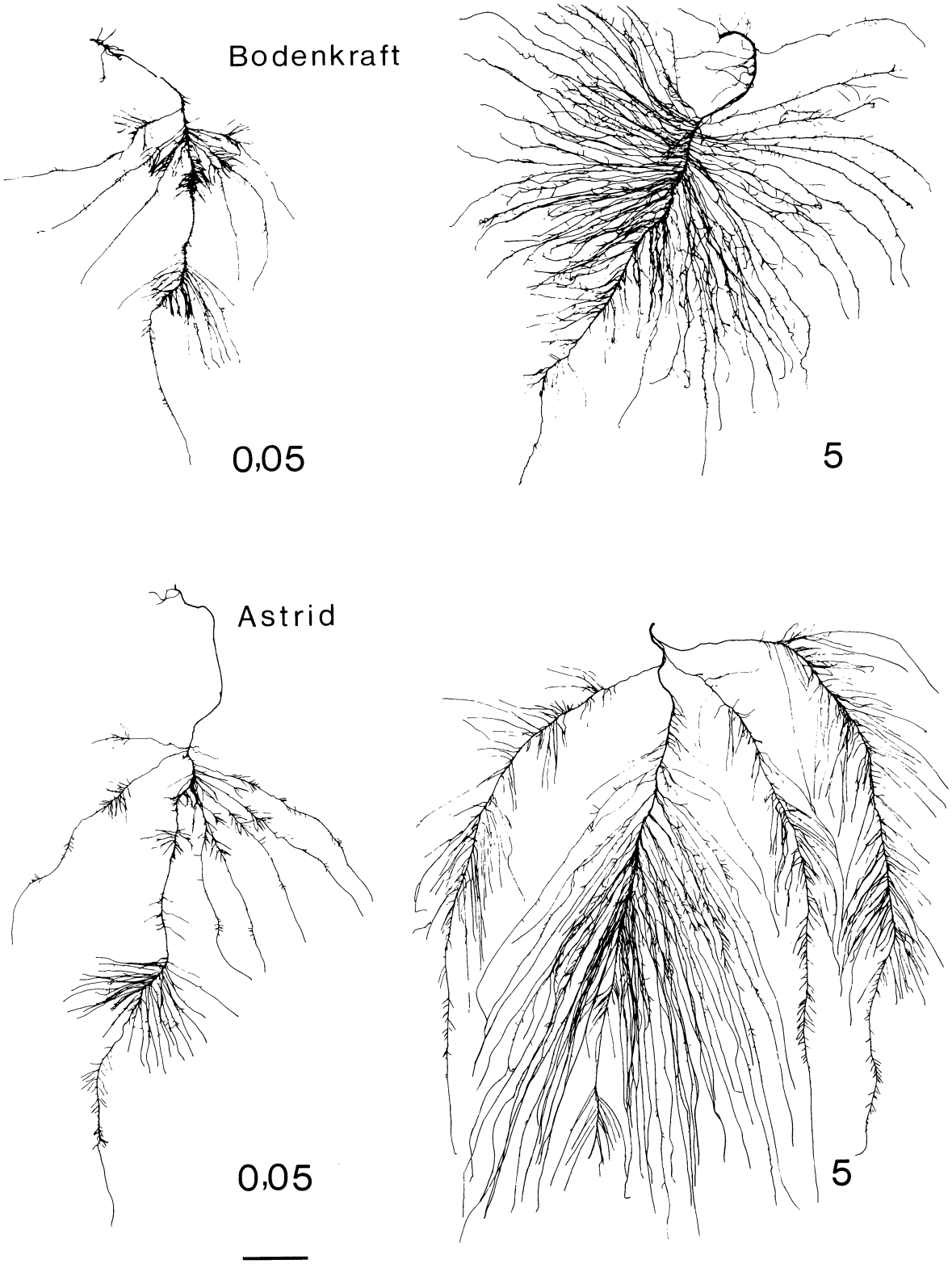

Fig. 4. Influence of NO_3^- concentration (mol m^{-3}) on root morphology of the varieties 'Astrid' and 'Bodenkraft'. *Bar* represents 5 cm.

Table 2. Influence of the NO_3- concentration on root dry weight, root volume, root surface area and specific root weight (length of 1 g root), of the varieties "Astrid" and "Bodenkraft"

N-level ($mol\,m^{-3}$)	Root parameters				
	Dry weight[a]	Volume[b]	Surface area[c]	Length[d]	Specific length[e]
"Astrid"					
0.05	0.45	4.8	63	67	148
0.5	1.39	28.8	314	277	225
5.0	1.82	42.5	577	502	284
LSD 5%	0.32	9.9	87	97	42
"Bodenkraft"					
0.05	0.16	1.8	21	30	187
0.5	0.74	10.3	119	109	147
5.0	1.08	14.5	171	163	150
LSD 5%	0.42	8.7	62	44	23

[a] dry weight expressed in g $plant^{-1}$.
[b] volume expressed in cm^3.
[c] surface area expressed in dm^2 $plant^{-1}$.
[d] length expressed in m $plant^{-1}$.
[e] specific length expressed in $m\,g^{-1}$ dry weight.

formation, leading to an increase in the relative root length, especially in 'Astrid'. The genotypical differences in root morphology, due to increasing nitrate concentrations, are particularly evident in the specific root length, which increases in 'Astrid' but is not significantly affected in 'Bodenkraft' (Table 2).

Discussion

There is general agreement that increasing nitrogen supply over a wide range of concentrations enhances total plant growth (Maizlisch *et al.*, 1980; Marriott and Dale, 1977; Tennant, 1976). Shoot growth, however, is stimulated more than root growth (Geisler and Krützfeld, 1984), leading to a negative correlation between nitrogen levels and root fraction (Fig. 1). At high nitrogen concentrations the size of the root system required to maintain maximum nitrogen absorption rate is reduced (Robinson and Rorison, 1983). Thus the decrease in root fraction may reflect a functional balance between root and shoot growth, as suggested by Brouwer, (1983) and Szaniawski, (1983).

Stimulation of root growth by increasing nitrogen levels (Table 2; Fig. 3) is mainly a result of lateral root formation, as has been shown also by Drew and Saker (1975) in barley with a localized nitrate supply. There are, however, remarkable genotypical differences in potato root response to increasing nitrate supply: 'Astrid' forms more and longer laterals than 'Bodenkraft' (Fig. 4).

The size of the root system is undoubtedly of great importance for the uptake of mineral nutrients with low mobility in the soil, such as phosphorus and potassium (Barber and MacKay, 1986; Silberbush and Barber, 1983). The situation appears to be less clear for mobile ions such as nitrate. According to simulation models, rooting density only becomes a rate limiting factor for nitrate absorption at very low nitrate concentrations (Burns, 1980; Robinson and Rorison, 1983). This view is apparently supported by the results of Norby et al. (1986). These results indicate that stimulation of root growth by CO_2 enrichment in the shoot environment stimulates phosphorus and potassium, but hardly the nitrate uptake, from a soil low in fertility. However, particularly under field conditions neither a 'mean' rooting density exists, nor is the nitrate concentration maintained at a high level throughout the growing season of agricultural crops. While in the topsoil at high rooting density inter-root competition for nitrate can be high, in the subsoil utilization of nitrate may be limited by rooting density (Burns, 1980). Higher rooting density in the subsoil is becoming

increasingly important in later stages of growth, when the nitrate in the topsoil is depleted. Under such conditions the nitrate uptake from the subsoil depends on root proliferation in the subsoil, and on nitrate supply by diffusion to the root surface (Strebel *et al.*, 1983). In agreement with this, a positive correlation exists in maize varieties between rooting density in the subsoil and the nitrate depletion in the subsoil (W J Horst, personal communication).

It is therefore suggested that the larger root system of 'Astrid' enables this variety to absorb more nitrate at limited supply, and to realize its optimum yield at lower soil nitrogen availability than 'Bodenkraft'. The differences between the two varieties in nitrogen efficiency (Hunnius, 1981) are therefore mainly due to the differences in root morphology in general, and root response to nitrogen in particular.

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PLSO PN 131

Phosphorus efficiency and phosphorus remobilization in two sorghum (*Sorghum bicolor* (L.) Moench) cultivars

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Key words: P efficiency, $^{32}P/^{33}P$ labelling, P redistribution, P solubility, sorghum genotypes

Abstract

With two sorghum cultivars differing in P efficiency a P uptake experiment ($^{32}P/^{33}P$ labelling) was carried out followed by a period of P deficiency. The tendency of the total P distribution and redistribution pattern was rather similar in both sorghum cultivars. Although in the cultivar with a greater P absorbing capacity per unit root weight a higher proportion of the P was found in the inorganic P soluble fraction this is not necessarily an indication of a higher vacuolar affinity for P. Under P deficiency in both cultivars a rapid decrease of the TCA soluble P fraction in the leaves was observed. Before complete exhaustion of this fraction the TCA insoluble P fraction was also markedly reduced. In the roots the total P content was maintained fairly constant with a distinct shift in favour of the insoluble fraction occurring during the period of P deficiency. It is assumed that in the P efficient sorghum cultivar producing more dry matter per increment of P absorbed, rather inherent growth promoting factors contribute to the intraspecific P efficiency by a stimulation of the intensity of P redistribution and thus compensate for the lower P absorbing capacity of its roots.

Introduction

Many attempts have been made to exploit genotypic differences in the acquisition and utilization of mineral nutrients in plants, particularly with respect to phosphorus (Fox, 1978; Gerloff and Gabelman, 1983; Saric, 1987). In general, an evaluation of P efficiency in plants should include efficient absorption, but also an efficient internal use under conditions of suboptimal P supply. The intraspecific P-efficiency may be a function of a genotypically different compartmentation (either physical or metabolic) and direct use after its initial translocation into the tissue. Elliott and Läuchli (1985) suggested that P partitioning between inorganic and organic forms is the major determinate. In addition, an assumed genotypically higher vacuolar affinity for inorganic P could also be detrimental to P remobilization in the plant. In previous experiments with barley (Wieneke, 1985) it was shown that P remobilization is not only a matter of senescence but occurred even in young non-matured tissues supporting the idea of Biddulph (1959), who suggested that a specific P fraction is cycled in the plant.

The objective of this study was to follow the pattern of the P redistribution in two sorghum genotypes with different capacities for absorbing phosphate and to relate the P remobilization to different soluble P fractions.

Materials and methods

Two sorghum (*Sorghum bicolor* (L.) Moench) cultivars (NB 9040 and SC 33-9-8-E, denoted NB and SC) were used, which had been shown by Furlani *et al.* (1984) to differ in P absorption per unit root weight (SC more efficient) and conversely in the dry matter production per unit of P absorbed (NB more efficient). The plants were grown in nutrient solution (as used by Clark 1982) containing initially 15 μM KH_2PO_4 under growth-room conditions (photo-period: 12 h; 25 $\pm$ 2°C; 65%

RH; light intensity 450 μE m^{-2} s^{-1} — dark period: 8 h 21 ± 2°C; 85% RH; 2 h light adaptation at start and end of the photoperiod). The experimental set-up is shown in Table 1. The plants were labelled immediately after endo-sperm removal and transfer of the seedlings to the nutrient solution with a constant specific radioactivity of 74 kBq ^{32}P per μmol P during the period of P supply. Three days before the first harvest (23rd day) the plants were labelled with ^{33}P in addition to the still constant specific radioactivity of ^{32}P. After the first harvest the remaining plants were grown without P-supply.

At harvest time the roots were rinsed with distilled water and the plants were separated into individual plant parts, the leaves including the leave sheaths. The roots and 4th and 5th leaves (denoted 4/5th) as well the 7th and 8th leaves (7/8th) were each placed into liquid nitrogen, freeze dried and then ground. Aliquots of 20 — 50 mg were extracted with a solution of 2% chloroacetic acid (TCA) after the method of Barr and Ulrich (1963). Time and volumes were modified and tested to result in a complete and reproducible extraction. The other plant material was dried (105°C) for dry weight determination and samples were dry ashed (550°C) in a muffle furnace. The ash was dissolved in an aliquot of 0.5 *N* HNO_3.

Due to the labelling of the seedlings immediately after germination it was possible to determine the P content on the basis of the specific activity. The small amount of seed borne inactive P (below 5% of the total) was neglected. Aliquots of the double labelled P solutions were placed into Instagel cocktail for liquid scintillation counting as described earlier (Wieneke, 1985). Slightly coloured samples of the extracted fractions were cleared by the addition of charcoal before counting. If necessary quench corrections were performed. The values given in the tables and figures are means of 4 replicates except of 5 at the first harvest date. Only in a few cases extremely deviating single values were rejected.

Table 1. Scheme of experimental set-up and sequence

1.–4. day:	Germination in vermiculite
Period of P supply:	
4.–11. day:	Endosperm removed, seedlings in 1/5 concentrated nutrient solution with 15 μM ^{32}P
11.–14. day:	Transfer to full nutrient solution with 50 μM ^{32}P
14.–23. day:	Exchange of nutrient solution (dto.) at 3 day intervals
20.–23. day:	Double labelling with ^{32}P and ^{33}P
Period of P deficiency:	
23.–32. day:	Full nutrient solution but without P, harvests at intervals of 3 days at day 23, 26, 29 and 32

Results and discussion

Probably due to the early endosperm removal and moderate P supply, minor symptoms of 'red speckling' were observed only sporadically in the cultivar SC, which is commonly sensitive to this (Furlani *et al.*, 1986). The total P uptake was about 280 μmol per plant for both cultivars from a total supply in the nutrient solution of 445 μmol. This resulted in an average P concentration in the shoots and roots of 0.45 and 0.66% respectively for the cultivar NB, and of 0.54 and 0.95% for cultivar SC. After 9 days of P deficiency the concentration in the shoots and roots dropped to 0.08% and 0.1% for NB and to 0.1% and 0.13% in SC, respectively (*cf.* Table 2).

For clarity the standard deviations are not included in the data presented in the following figures. There was, however, considerable variation of the data, somewhat more in the cultivar SC (Table 2). An explanation is partly to be seen in the expression of the results on a per plant part basis, but presumably an enhanced variability of growth after endosperm removal and a rather limited amount of plants for selection after the germination has also contributed. Nevertheless, the pattern shown in the sequential harvests demonstrates well the tendency of P distribution and P redistribution in the two sorghum cultivars NB and SC: under moderate P supply the distribution pattern of P taken up during the entire period of P supply as compared to that absorbed during day 20 — 23 before harvest does not substantially differ nor between the two cultivars either (Fig. 1). One reason may be a relatively high ^{33}P uptake rate because of the fast growth of the plants during the last 3 days before harvesting. In principle, our results are in agreement with the tendency of previous experiments (Huckfeld, 1986), and those

Table 2. Dry weight and P content in shoots and roots expressed per plant of two different sorghum cultivars supplied with P until 1st harvest (day 23) followed by a 9 day period of P deficiency

Cultivar	Plant part	Day: 23	26	29	32
Dry weight (g)					
SC	Shoot	0.68 ± 0.2	1.11 ± 0.4	2.85 ± 0.1	3.15 ± 0.9
	Root	0.48 ± 0.2	0.78 ± 0.3	1.95 ± 0.2	2.18 ± 1.7
NB	Shoot	1.01 ± 0.2	1.92 ± 0.3	3.45 ± 0.3	5.24 ± 0.1
	Root	0.56 ± 0.1	1.13 ± 0.2	1.86 ± 0.2	4.16 ± 0.8
P (mg)					
SC	Shoot	6.16 ± 0.7	5.31 ± 1.1	6.08 ± 0.3	3.92 ± 1.0
	Root	2.45 ± 0.2	2.13 ± 0.5	2.67 ± 0.2	2.96 ± 1.4
NB	Shoot	6.36 ± 0.6	6.63 ± 0.4	6.09 ± 0.3	4.98 ± 0.3
	Root	2.48 ± 0.3	2.00 ± 0.2	2.35 ± 0.2	3.44 ± 0.8

obtained by Furlani *et al.* (1984), in that the two sorghum cultivars differ in P absorption per unit root (Fig. 2) and conversely in dry matter production per increment of P absorbed (Table 2).

During the period of P deficiency in both sorghum cultivars P is soon withdrawn, not only from the senescent leaves but also from the non-matured 4/5th leaves. Obviously, this P is trans-

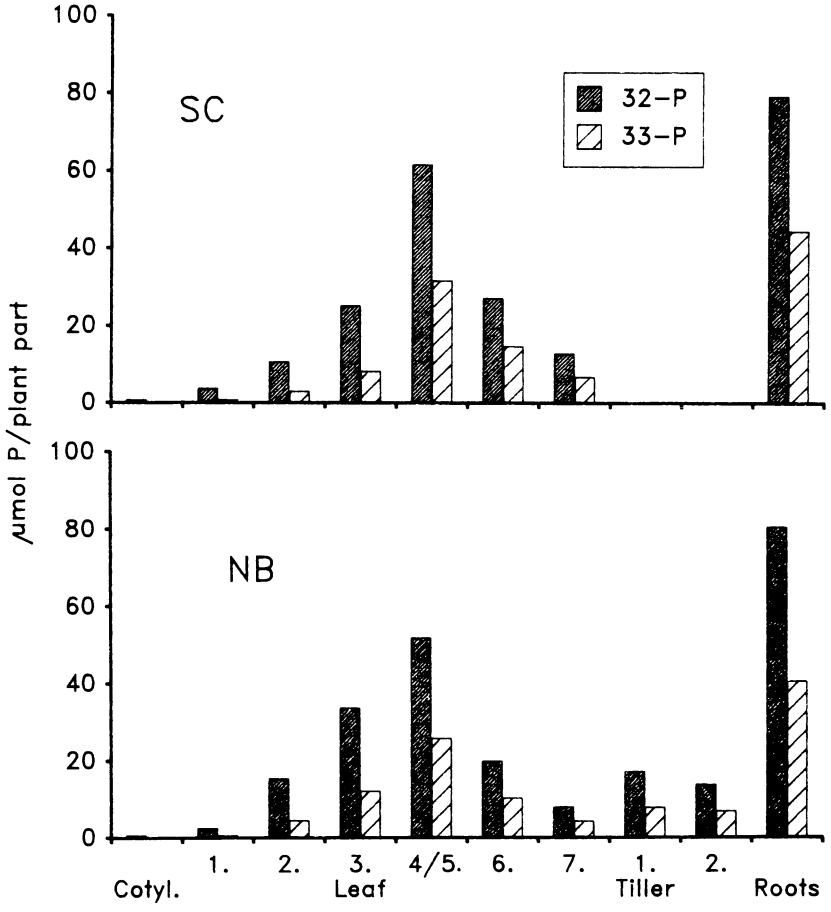

Fig. 1. Distribution pattern of P taken up by two sorghum cultivars during the total period of P supply (^{32}P: day 4–23) or during the last 3 days (^{33}P: 20–23).

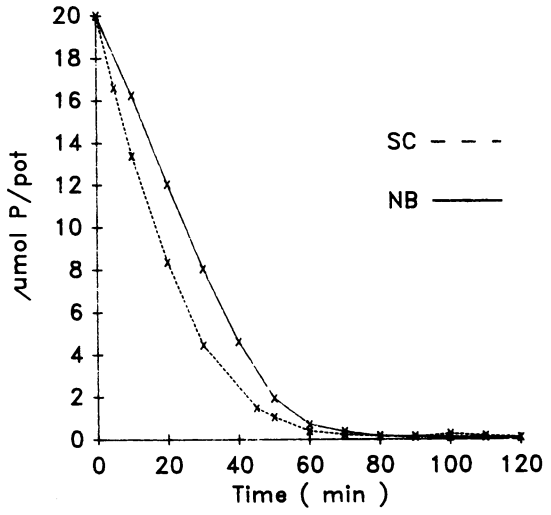

Fig. 2. Depletion of P in the nutrient solution by two sorghum cultivars: 0.8 l; 5 plants, ^{32}P labelling.

ferred to the very young fast growing 6th leaf and later to the newly developing 7/8th leaves (Fig. 3). Although the dry weight of the 4/5th leaves increased by 40—50% during the 9 days of P deficiency (Fig. 4) about 70–80% of the P is withdrawn; similar relationships exist in the 7/8th leaves after the 3rd and 4th harvest (data not shown). In contrast, in the roots the P content is maintained or seems rather to be enlarged in relation to the increase in the total dry matter (Table 2).

Due to the constant specific radioactivity applied during the P uptake it was possible to determine even very small P amounts in different soluble fractions. Despite the similarities in the total P distribution and redistribution patterns, between the two sorghum cultivars distinct differences exist in the P solubility (Fig. 5). The relatively high P absorbing efficiency of the cultivar SC (Fig. 2, Table 2) is correlated with higher amounts of P in

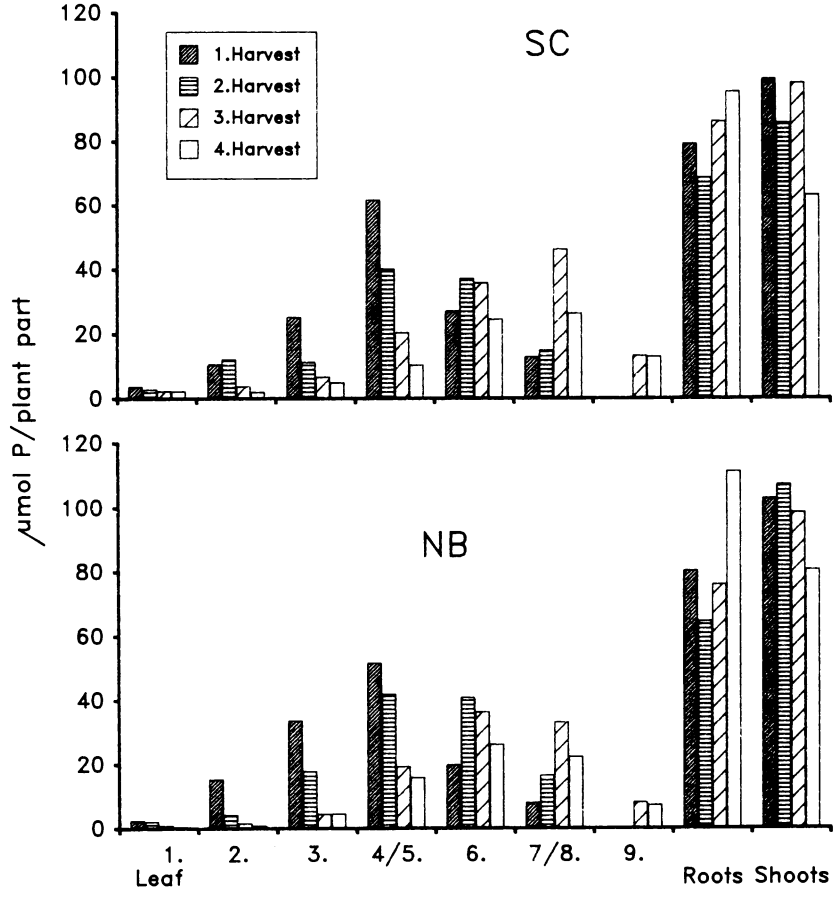

Fig. 3. Changes of P distribution at 3 day intervals of P deficiency in two sorghum cultivars. 1. harvest date: P distribution after period of P supply.

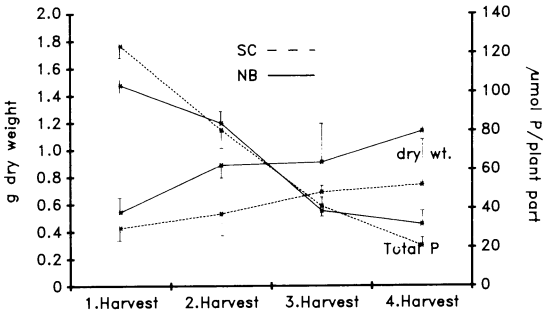

Fig. 4. Loss of P and dry weight increase in the 4/5th leaves of two sorghum cultivars during a 9 day period of P deficiency.

the soluble fractions of the extracted 4/5th leaves. Nevertheless, in both cultivars the amount of P in the soluble fractions is already much reduced after 3 days of P deficiency in the root medium (Fig. 5), whereas the P content of the insoluble P fraction remains first of all nearly constant. This rapid withdrawal of P from the inorganic and organic soluble fractions in the still growing 4/5th leaves may not be interpreted as due to the effects of a strong vacuolar affinity as suggested by Elliot and Läuchli (1985) to function in maize, and thus is not an appropriate explanation for the differing P efficiency between the two sorghum cultivars. On the other hand, at the 3rd and 4th harvest the amount of P in the insoluble fraction drops even before the soluble P fractions are reduced to a comparable level.

However, to obtain more specific information concerning the sites and developing gradients of P changes in the tissue, a much more detailed fractionation of the tissue of a single leaf would be

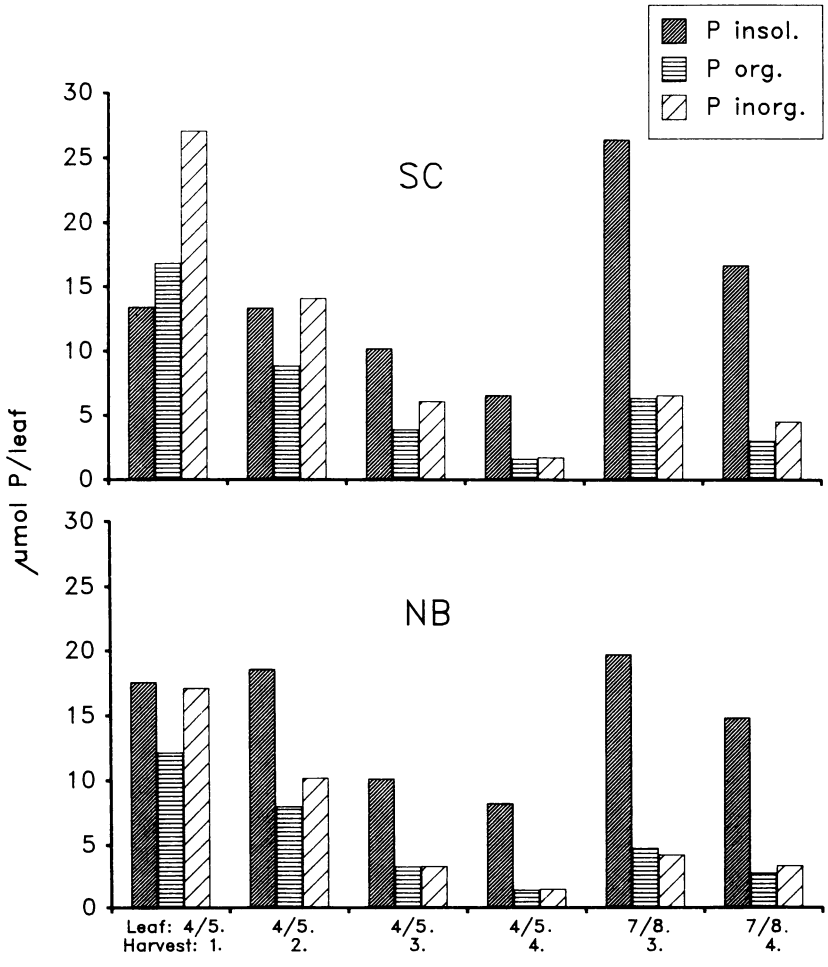

Fig. 5. Changes of P content in different fractions of young leaves at 3 day intervals of P deficiency in two sorghum cultivars. 1. harvest date: P distribution after period of P supply.

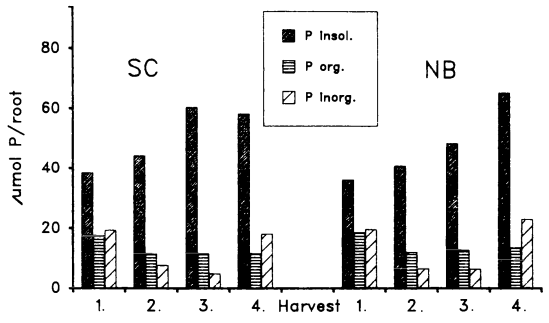

Fig. 6. Changes of P content in different P fractions of the roots at 3 day intervals of P deficiency in two cultivars. 1. harvest date: P distribution after period of P supply.

necessary. Moreover, it is still a matter of question to what extent in a non-matured leaf with a P import and a simultaneous P export (Wieneke, 1985) starting at the distal regions the retranslocated P will bypass the growing tissue at the base (Moorby, 1981). Furthermore, the substantial P removal from the inorganic soluble P fraction and the change in the P distribution already after 3 days of withholding P should be noted with respect to many transient P-starvation experiments reported in the literature (Clarkson, 1981) when no growth alteration is considered.

In comparison to the leaves, the roots behave quite differently in the two sorghum cultivars (Fig. 6). There is a remarkable accumulation of P in the insoluble fraction at the harvest date after the period of P supply, and this pattern becomes even more distinct during the period of withholding P. A similarly preferential accumulation of P in the insoluble P fraction is also observed in the very young leaves emerging under P deficiency (Fig. 5). This accumulation in the TCA-insoluble P compounds may indicate that P in nucleic acids, proteins and lipids (Barr and Ulrich, 1963) represents a most important fraction for cell growth and cell functioning.

The maintenance or even increase of the total P content in the roots in a period of P starvation might be related to an enhanced change of the assimilate distribution in favour of the roots in plants under P stress (Burauel *et al.*, 1989). P is known to be translocated in the phloem together with phosynthates towards a sink region. Whether the removal of P from the 4/5th leaves during the P deficiency impaired the capacity of the photosynthesis (Whiteacker *et al.*, 1976) is beyond the scope of this investigation. Despite the severe drop in the average P content to a level below about 0.1% during the 9 days of P deficiency no necrosis was observed and there was still some growth of the shoots and roots (Table 2). In agreement with the conclusions of Chapin and Bieleski (1982), it is suggested that there are rather inherent growth promoting factors which substantially contribute to the intraspecific P efficiency of the cultivar NB by a stimulation of the intensity of the P redistribution and thus compensate for the better P acquisition by the root of the sorghum cultivar SC.

Acknowledgements

The author is grateful to Mrs C Huckfeldt and Mr U Weller for competent technical assistance.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 73–76.
PLSO PN 132
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Responses to phosphate fertilizers of differing solubilities by white clover cultivars

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Key words: cultivars, monocalcium phosphate, partially acidulated phosphate rock, phosphate response, reactive phosphate rock, *Trifolium repens*, white clover

Abstract

It is well documented that plant species differ in their ability to utilise phosphorus (P) from phosphatic fertilizers of differing water solubility. However, evidence for intraspecific differences is less available. The present study examined the response of four morphologically distinct white clover (*Trifolium repens* L.) cultivars, with diverse genetic backgrounds, to three contrasting phosphatic fertilizers. The cultivars Barbian, Grasslands Tahora, Grasslands Huia and Grasslands Kopu were grown in soil in pots with P supplied as either monocalcium phosphate (MCP), partially acidulated phosphate rock (PAPR) or North Carolina phosphate rock (NCPR), at seven P rates ranging from 0 to 700 mg P kg^{-1} of soil. Measurements were made of leaf number and weight, stolon length and weight, and root length and weight.

There was significant variation between cultivars in their leaf dry weight, shoot dry weight, proportion of stolon and root length response to P when supplied as different forms of P. Kopu, a large leaved cultivar, responded more rapidly in the upper part of the response curve than Barbian, Tahora or Huia when supplied with P in the form of MCP and NCPR, and reached maximum yield at a lower level of added P.

Introduction

Plant species differ in their ability to obtain phosphorus (P) from relatively insoluble phosphate rock materials (Aguilar and van Diest, 1981; Drake and Steckel, 1955; Fried, 1953; Kerridge and Ratcliffe, 1982; Tennant, 1975). Species such as buckwheat and rape absorb more phosphate from phosphate rocks than legumes such as lucerne, red clover and white clover which, in turn, absorbed more than grasses such as ryegrass, oats, maize and wheat. However, evidence for intraspecific differences is less available. Small differences in the ability of *Stylosanthes* sp. accessions to absorb P from phosphate rock have been observed (Jones, 1974). Six carrot cultivars were unable to utilise phosphate rock when grown in cell culture, although five were shown to have some ability to do so as intact plants (Olsen *et al.*, 1954). No differences were found between two cultivars of tomato for P uptake and dry weight production when supplied with different phosphate rocks (Hernandez and Sartani, 1984).

In recent years, there has been considerable change in the New Zealand fertilizer industry from the traditional single superhosphate (SSP) to less processed, higher P analysis materials, including partially acidulated phosphate rock (PAPR) and reactive phosphate rock such as North Carolina phosphate rock (NCPR). Phosphorus is applied to pastures in New Zealand primarily to stimulate white clover (*Trifolium repens* L.) growth which in turn increases nitrogen input, through nitrogen fixation, and hence grass growth. The present study aimed to determine the extent of intraspecific variation amongst four morphologically distinct white

clover cultivars with diverse genetic backgrounds for their response to three phosphate fertilizers of differing solubility.

Method

Soil

The soil used in this study was the Wainui silt loam (Typic Dystrochrept), with a pH of 5.0 and Olsen P of 6 mg kg^{-1}. It was collected from the field to a depth of 100 mm and then sieved moist to pass a 4-mm sieve.

Phosphate fertilizer

Monocalcium phosphate (MCP) was used to represent SSP. The PAPR was made by acidulating 30% of total P of NCPR with phosphoric acid. Total P in MCP, PAPR and NCPR was 25, 15 and 13%, and water soluble P was 90, 50 and < 0.1% of total P, respectively. The MCP was powdered, PAPR granulated and NCPR had 100% < 250 μm.

Plant material

Seeds of cultivars Grasslands Kopu, Grasslands Tahora, Grasslands Huia and Barbian (Caradus, 1986) were germinated at 25°C, in petri-dishes containing moist filter paper, and then transplanted after 48 h to sand trays. Seedlings were grown in sand for 10 days before planting into pots.

Glasshouse study

Each P fertiliser was added to soil at rates of 0, 35, 75, 150, 300, 450 and 700 mg P kg^{-1} of soil. Sulphur as gypsum ($CaSO_4$) and potassium as potash (KCl) was added as a dry mix along with the fertiliser to soil. Oven dried soil (572 g) was packed into 650 ml pots, watered to field capacity and incubated for 30 days in the glasshouse before transplanting four seedlings from sand trays into each pot. Each treatment was replicated three times and pots were arranged in a randomised block design. Pots were watered daily by weight to field capacity, a gravimetric moisture content of 53%. A *Rhizobium trifolii* inoculum cocktail containing strains NZP540, 541, 547, 548 and 560 was added to each pot one week after planting.

Measurements

A destructive harvest was taken 65 d after planting and measurements were made of leaf number and weight, stolon length and weight, and root length (Syers and Mackay, 1986) and weight. Number of plants surviving was recorded. Estimates of plant available P (Kerridge and Ratcliffe, 1982) in soil were also made at the end of the study.

Statistical analyses

All data were calculated on a per pot basis and subjected to an analysis of variance. In all cases, except for proportional data, it was necessary to log-transform data to satisfy assumptions made in analysis of variance. Log-transformation of data for dry weight also provided a means of distinguishing between cultivar × P level or cultivar × P source interactions caused by differences between cultivars in the uptake and use of P from those caused simply by growth rate. Antonovics *et al.* (1966) argue that a fast growing cultivar would be expected to have a greater response to P than a slower growing cultivar. Assuming exponential growth, the slope of log-yield against time would be the growth rate, and the intercept of the line with the vertical axis would represent the starting weight. The absence of a differential response between two varieties after log-transformation of data would suggest that the growth rate has been changed equally in both varieties by the nutrint treatments.

Results and discussion

Morphological characteristics of cultivars

Barbian had more leaves than Huia and Kopu, but like Tahora the leaves were much smaller than

Table 1. Effect of cultivar on shoot and root characters. Each value is the mean of seven P levels and three P sources

Cultivar	Log-total dry weight (g)	Proportion			Log-individual leaf weight (g)	Log-leaf number	Log-stolon length (cm)	Log-root length (cm)
		Leaf	Stolon	Root				
Barbian	0.664	0.64	0.19	0.17	0.005	4.0	2.80	6.66
Tahora	0.662	0.63	0.15	0.22	0.006	3.8	2.56	6.94
Huia	0.730	0.65	0.13	0.22	0.009	3.6	2.40	7.07
Kopu	0.912	0.65	0.13	0.23	0.013	3.6	2.43	7.62
p	***		***	***	***	***	**	***
$LSD_{0.05}$	0.140	NS	0.02	0.02	0.001	0.3	0.35	0.40

those for either Huia or Kopu (Table 1). Fewer large leaves, as in the case of Kopu, appears to be a more effective combination for producing dry matter than a larger number of small leaves as in the case of Barbian. Barbian partitioned more dry matter to stolon, but less to root than the other 3 cultivars (Table 1). This was also reflected in root lengths.

Effect of P source on cultivar (cultivar × P source interactions)

There were no significant cultivar × P source interactions. A previous study comparing the growth of five white clover populations on sodium phosphate (NaH_2PO_4 $2H_2O$), Gafsa phosphate rock and aluminium phosphate ($AlPO_4$) supplied at a single rate of 50 kg P ha^{-1} also found no significant P source × population interaction (Caradus, 1984). Furlani *et al.* (1987) working with 10 sorghum genotypes recorded no genotype × P source interactions for the growth and P uptake of these genotypes on potassium, calcium, ferric and aluminium phosphate supplied at 56 kg ha^{-1}.

Cultivar responses to P sources (cultivar × P level × P source interactions)

Differences between cultivars in response to added P were significantly affected by the three P sources for log leaf dry weight ($p < 0.05$), log shoot dry weight ($p < 0.05$) and log root length ($p < 0.05$).

Analysis by cultivar showed that P level × P source interactions for log shoot dry weight were significant ($p < 0.05$) for Barbian, Tahora and Kopu but not for Huia. Huia had similar response characteristics, irrespective of P source, whereas Barbian, Tahora and Kopu responded more at high P additions when supplied with MCP than PAPR or NCPR.

Analysis by P source showed that P level × cul-

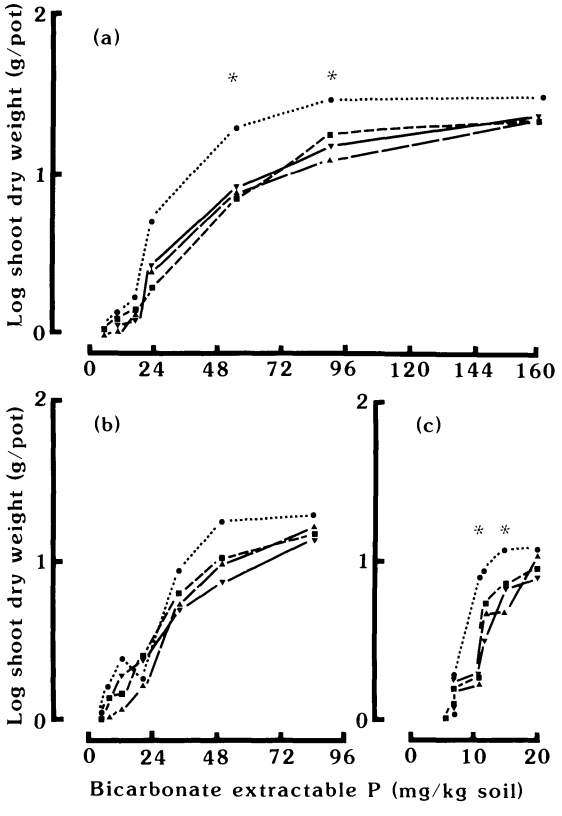

Fig. 1. Log shoot dry weight response of Barbian (▼——▼), Tahora (▲— —▲), Huia (■— — —■) and Kopu (● · · · · · · · · · ·●) to (**a**) MCP, (**b**) PAPR and (c) NCPR. *Plotted values* at each P level are means of three replicates.

tivar interactions for log shoot dry weight were significant ($p < 0.05$) when supplied with P in its most soluble form, MCP and the least soluble form, NCPR (Fig. 1). These two P sources behave very differently in soils (Sidwell and Krul, 1978). This is reflected in the marked difference in the amounts of bicarbonate extractable P in the soil following the addition of these two P sources. Kopu responded more rapidly to both MCP and NCPR in the upper part of the response curve and reached a maximum yield at a much lower level of added P than Barbian, Huia or Tahora. The absence of cultivar differences when supplied with P in the form of PAPR might reflect the fact that the PAPR granules were only lightly ground before addition to soil, while MCP and NCPR were added in the powdered form. Addition of small granules of PAPR to soil might have reduced, by localisation, the plant available P fraction from this P source, sufficient to limit the potential growth of Kopu.

Conclusion

The P response characteristics of the cultivars varied significantly with P source. Kopu, a large leaved cultivar responded more rapidly to P in the mid to upper part of the response curve than Barbian, Tahora or Huia when supplied with P as MCP and NCPR and reached maximum yield at a lower level of added P.

Acknowledgement

To Allison MacKay and Miranda Filemoni for technical assistance, and to Cherry Liddane for supplying Rhizobium inoculum.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 77–83.
PLSO PN 133
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Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores

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Key words: barley, chlorosis resistance, cucumber, genotypical differences, grasses, iron mobilization, iron uptake, maize, microorganisms, oat, phytosiderophores, rice, root exudates, root growth, rye, sorghum, wheat

Abstract

Graminaceous species can enhance iron (Fe) acquisition from sparingly soluble inorganic Fe(III) compounds by release of phytosiderophores (PS) which mobilize Fe(III) by chelation. In most graminaceous species Fe deficiency increases the rate of PS release from roots by a factor of 10–20, but in some species, for example sorghum, this increase is much less. The chemical nature of PS can differ between species and even cultivars.

The various PS are similarly effective as the microbial siderophore Desferal (ferrioxamine B methane sulfonate) in mobilizing Fe(III) from a calcareous soil. Under the same conditions the synthetic chelator DTPA (diaethylenetriamine pentaacetic acid) is ineffective.

The rate of Fe(III)PS uptake by roots of graminaceous species increases by a factor of about 5 under Fe deficiency. In contrast, uptake of Fe from both synthetic and microbial Fe(III) chelates is much lower and not affected by the Fe nutritional status of the plants. This indicates that in graminaceous species under Fe deficiency a specific uptake system for FePS is activated. In contrast, the specific uptake system for FePS is absent in dicots. In a given graminaceous species the uptake rates of the various FePS are similar, but vary between species by a factor of upto 3. In sorghum, despite the low rate of PS release, the rate of FePS uptake is particularly high.

The results indicate that release of PS and subsequent uptake of FePS are under different genetic control. The high susceptibility of sorghum to Fe deficiency ('lime-chlorosis') is most probably caused by low rates of PS release in the early seedling stage. Therefore in sorghum, and presumably other graminaceous species also, an increase in resistance to 'lime chlorosis' could be best achieved by breeding for cultivars with high rates of PS release. In corresponding screening procedures attention should be paid to the effects of iron nutritional status and daytime on PS release as well as on rapid microbial degradation of PS.

Introduction

Iron (Fe) deficiency chlorosis ('lime chlorosis') is a widespread mineral nutrient deficiency in higher plants grown on calcareous soils (Vose, 1982). Soil amendments and foliar sprays of Fe are not viable economically in the long term to correct Fe deficiency. Therefore, to alleviate Fe chlorosis in the long term, alternative methods, for example breeding of more chlorosis resistant genotypes (cultivars) are required.

Besides Fe inactivation in plants (Mengel and Geurtzen, 1986), insufficient mobilization in the rhizosphere and uptake of Fe are the main causes for Fe chlorosis in plants growing on calcareous soils. Accordingly, chlorosis resistance correlates closely with quantitative differences in Fe acquisition (Marschner *et al.*, 1986; Römheld, 1987).

In contrast to all other plant species, graminaceous species (grasses) have a different mechanism for Fe acquisition. The mechanism in grasses is characterized by two main components:

1) Fe deficiency-induced increase in release of Fe(III) mobilizing (chelating) root exudates (phytosiderophores, PS) and 2) a highly efficient uptake system for Fe(III)PS which is further activated under Fe deficiency (Marschner *et al.*, 1986; Römheld, 1987; Takagi *et al.*, 1984). Both components of this mechanism are located in apical root zones (Marschner *et al.*, 1987). Therefore, not only the degree of Fe deficiency but also the rate of root growth shall effect Fe acquisition.

It was the aim of this study to determine in graminaceous species, with differences in chlorosis resistance the rate of PS release, their chemical nature, and the uptake rates of different FePS in relation to the Fe nutritional status of the plants.

Material and methods

The following graminaceous species were precultured in nutrient solution: *Avena sativa* L. cv. Pirol; *Hordeum vulgare* L. cvs. Europa, Rikuzen-Mugi and Minori-Mugi; *Oryza sativa* L. cv. unknown; *Secale cereale* L. cv. Merkator; *Sorghum bicolor* (L.) Moench cvs. SPV-393, MS 35-1, and one cultivar of sudan grass [also *S. bicolor* (L.) Moench]; *Triticum aestivum* L. cv. Okapi; *Zea mays* L. cvs. Garbo and Mutin. Plants were grown in a continuously aerated nutrient solution of the following composition (m*M*): $Ca(NO_3)_2$, 2.0; K_2SO_4, 0.7; $MgSO_4$, 0.5; KH_2PO_4, 0.1; KCl, 0.1; trace elements (μM): H_3BO_3, 10; $MnSO_4$, 0.5; $ZnSO_4$, 0.5; $CuSO_4$, 0.2; $(NH_4)_6Mo_7O_{24}$, 0.01. Fe adequate plants were grown with 0.1 m*M* FeEDTA. 100 plants per 5 L and 9 plants per 1.5 L plastic pots were used for the experiments to measure the rates of release of phytosiderophores (PS) and uptake of ^{59}FePS, respectively. Preculture and experiments were performed in a climate chamber (16/8 h light/dark, 40 Wm^{-2} fluorescent lamps, 25/23°C, 60–75% rel. humidity).

Root exudates (phytosiderophores) were collected according to Awad *et al.* 1988, purified and separated by ion exchange chromatography (Takagi *et al.*, 1984). The amount of released PS were indirectly determined by the amount of Fe(III) mobilized from Fe hydroxide (Marschner *et al.*, 1987). The purified PS of the various genotypes have been identified by a HPLC method (Kawai *et al.*, 1987) with reference substances of mugineic acid (MA), 2′deoxymugineic acid (DMA), epi-3 hydroxymugineic acid (HMA) and avenic acid (AA).

Solubilization of Fe from a ^{59}Fe labelled calcareous soil ($CaCO_3$ 14.8%, pH 8.6, organic matter 0.84%, CEC 9.9 mequiv. 100 g^{-1} soil, DTPA extractable Fe 2.52 mg kg^{-1} soil) by various chelators was determined in a chelating resin/dialysis tube system (Awad *et al.*, 1988).

Preparation of ^{59}Fe labelled Fe chelates and uptake experiments were performed according to Marschner *et al.* (1987). The PS used in the uptake experiments were collected from Fe deficient plants of the following species: MA, barley cv. Minori-Mugi; HMA, barley cv. Europa; DMA, barley cv. Rikuzen-Mugi; AA, oat cv. Pirol; unknown PS from sorghum cv. SPV-393. At the end of the uptake period extracellular Fe was removed from the roots and ^{59}Fe determined by liquid scintillation counting (Marschner *et al.*, 1987).

Each experiment was performed with 4 replicates.

Results

Release of phytosiderophores (PS)

The rate of PS release by the various genotypes is enhanced under Fe deficiency by a factor of 10–20, sorghum being an exception (Table 1).

Table 1. Release of phytosiderophores (PS) by Fe sufficient (+ Fe) and Fe deficient (− Fe) graminaceous species. Plant age 19 days, release of PS into nutrient solutions, non-sterile conditions, 25°C

Species, cultivar	Rate of release (μmol g^{-1} root DW d^{-1}) preculture	
	+ Fe	− Fe
Hordeum vulgare, Europa	0.4 ± 0.1	8.2 ± 1.2
Hordeum vulgare, Rikuzen-Mugi	n.d.[a]	6.3 ± 1.1
Hordeum vulgare, Minori-Mugi	n.d.	3.9 ± 0.6
Triticum aestivum, Okapi	0.5 ± 0.2	7.5 ± 0.8
Avena sativa, Pirol	0.3 ± 0.1	3.8 ± 0.6
Secale cereale, Merkator	0.3 ± 0.2	2.7 ± 0.5
Zea mays, Garbo	0.2 ± 0.1	2.0 ± 0.4
Sorghum bicolor, SPV-393	0.2 ± 0.1	0.4 ± 0.2
Sorghum bicolor, MS 35-1	n.d.	0.3 ± 0.1
Sorghum bicolor, Sudangrass	n.d.	1.2 ± 0.3

[a] not determined.

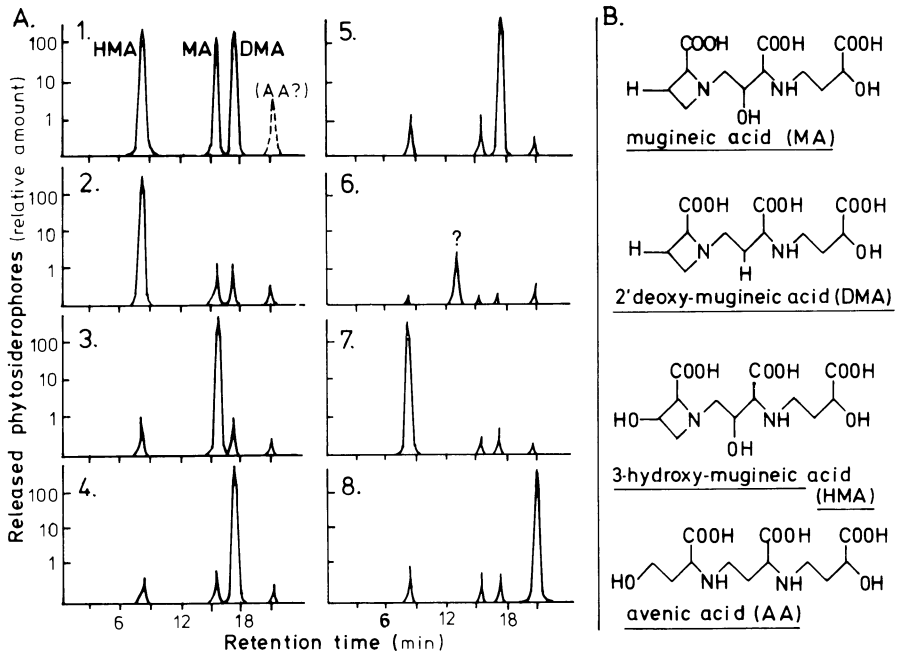

Fig. 1. **A.** Chromatograms (modified HPLC method, Kawai *et al.*, 1987) of phytosiderophores in root exudates from various graminaceous species/cultivars. 1. mixture of reference substances: mugineic acid (**MA**); 2'deoxymugineic acid (**DMA**); 3 hydroxymugineic acid (**HMA**); 2-4. *Hordeum vulgare* cvs. Europa, Minori-Mugi, Rikuzen-Mugi, respectively; 5. *Triticum aestivum* cv. Okapi; 6. *Sorghum bicolor* cv. SPV-393; 7. *Secale cereale* cv. Merkator; 8. *Avena sativa* cv. Pirol.
B. Molecular structure of MA, DMA, HMA, and AA (Avenic acid).

Among the genotypes tested, under Fe deficiency the rates of PS release decrease in the following sequence: barley > wheat > rye > maize ≫ sorghum.

The chemical nature of the PS differ in part between plant species and even cultivars within a species (Fig. 1). In all instances one PS dominates in each genotype. The PS were characterized by comparison with reference substances of MA, DMA and HMA. Therefore, in some instances the identification of the PS released could not be made with complete certainty.

Table 2. Mobilization of Fe from a calcareous soil by different ferric chelators. The soil was labelled with ^{59}Fe

Chelator (10^{-5} *M*)	Fe mobilization (nmol $^{59}Fe\ g^{-1}$ soil $12\,h^{-1}$)
Control (10^{-4} *M* $CaSO_4$)	1.0 ± 0.2
Phytosiderophore (HMA)	23.6 ± 1.3
Microbial chelator (Desferal)	19.2 ± 1.4
Synthetic chelator (DTPA)	2.0 ± 0.3

Mobilization of Fe from a calcareous soil

The ability of PS in comparison to other chelators, to mobilize Fe from a calcareous soil is shown in Table 2. Both the PS and the microbial siderophore were similarly effective in mobilizing Fe whereas the synthetic chelator was nearly ineffective under these conditions.

Uptake rate of Fe

The uptake rate of Fe supplied as FePS depends on the Fe nutritional status of the plants as shown in Table 3 for barley. Iron deficient plants (−Fe) were characterized by lower concentrations of chlorophyll (mild chlorosis) and Fe in the shoots and the highest Fe uptake capacity. In plants pretreated with FeEDTA the concentrations of chlorophyll and Fe were higher and Fe uptake capacity lower. Pretreatment with FePS had a similar effect as FeEDTA on chlorophyll concentrations, but further increased Fe concentrations

Table 3. Effect of the Fe nutritional status of barley on chlorophyll and Fe content in the shoot and on the uptake rate of Fe supplied as Fe phytosiderophores (0.1 μM ^{59}Fe HMA)

Preculture	Chlorophyll (mg g^{-1} dwt)	Total Fe (μg g^{-1} dwt)	HCl (0.5 M) extractable Fe (μg g^{-1} dwt)	Fe uptake rate (nmol g^{-1} root dwt 4 h^{-1})
−Fe	7.5 ± 0.9	64 ± 12	41 ± 8	595 ± 99
FeEDTA 0.1 mM	12.3 ± 1.3	90 ± 16	65 ± 10	405 ± 46
FeEDTA + Fe phytosiderophores (0.1 mM)[a]	12.8 ± 1.2	328 ± 33	260 ± 22	115 ± 15

[a] non-labelled Fe phytosiderophores were supplied 3 days prior uptake experiment.

distinctly lowered Fe uptake capacity. Thus, the Fe concentrations in the plants are better correlated with the capacity for Fe uptake from FePS than the chlorophyll concentration.

Evidence for the existence of a highly specific uptake system for FePS in graminaceous species is presented in Table 4. Compared to the uptake rate of FePS the uptake rate of Fe from either synthetic or microbial chelates is lower by a factor of 200–1000 and not affected by the Fe nutritional status. The uptake rate of FePS by sorghum remained higher even after FeEDTA pretreatment in combination with Fe citrate foliar spray. Depression of FePS uptake rate in this species required pretreatment with FePS.

The uptake rate of the different FePS were similar within any given species but varied by a factor of upto 4 between species (Table 5). These differences between species appear to be related to differences in root morphology. The highest uptake rates per unit root dry weight were found in Fe deficient rice and sorghum which are characterized by a fine highly branched root system. The lowest uptake rates were found in maize which has relatively thick roots.

The principal differences in the mechanism for Fe uptake between graminaceous species and other plant species are shown in Table 6 for barley and cucumber as representative examples. Synthetic chelate and FePS are both suitable sources of Fe for cucumber whereas for barley, the synthetic chelate is a poor source of iron for Fe deficient as well as Fe sufficient plants.

Discussion

Graminaceous species and varieties within a species differ considerably in their chlorosis resistance under field conditions (McDaniel and Brown,

Table 4. Effect of the Fe nutritional status of barley, oat, and sorghum plants on uptake rate of Fe (^{59}Fe) supplied as different chelates in nutrient solution (plant age 15 days, ^{59}Fe concentration 1 μM, pH 6.5)

Plant species cultivar	Precultur +/− 0.1 mM chelate	^{59}Fe uptake rate (nmol g^{-1} root dwt 4 h^{-1}) Fe supplied as		
		Synthetic Fe chelate (FeEDDHA)	Microbial Fe chelate (Fe Desferal)	Fe phytosiderophore (FeHMA)
Avena sativa cv. Pirol	−Fe	0.32 ± 0.09	n.d.	3826 ± 520
	FeEDTA[a]	0.34 ± 0.11	n.d.	450 ± 73
Hordeum vulgare cv. Europa	−Fe	0.51 ± 0.12	1.21 ± 0.24	3456 ± 473
	FeEDTA[a]	0.38 ± 0.09	2.50 ± 0.31	563 ± 99
Sorghum bicolor cv. SPV-393	−Fe	0.79 ± 0.15	2.61 ± 0.36	4935 ± 513
	FeEDTA[a]	0.74 ± 0.13	n.d.	3974 ± 461
	FeEDTA + Fe phytosiderophore[b]	n.d.	n.d.	776 ± 108

[a] Plants received daily 0.1% (w/v) Fe citrate foliar application in addition to FeEDTA in nutrient solution.
[b] Non-labelled FePS were supplied 2 days prior uptake experiment.

Table 5. Uptake rate of Fe in Fe deficient graminaceous species supplied with different Fe(III) phytosiderophores (plant age 15 days, Fe concentration 1 μM, pH 6.5). Each value represents the average of 3 replicates with a standard deviation (SD) between 2 and 16%

Plant species, cultivar	^{59}Fe uptake rates (μmol g^{-1} root dwt 4 h^{-1}) Fe supplied as				
	MA	DMA	HMA	AA	PS[a]
Avena sativa, Pirol	4339	4618	3826	4380	n.d.[b]
Hordeum vulgare, Europa	3807	3769	3456	3616	3758
Oryza sativa	6440	6798	5891	5987	n.d.
Secale cereale, Merkator	3085	2817	3117	3331	n.d.
Sorghum bicolor, SPV-393	5124	5429	4935	4423	4880
Triticum aestivum, Okapi	3278	3178	2995	3189	n.d.
Zea mays, Mutin	1622	2016	1673	2076	n.d.

[a] Unknown phytosiderophores released by Fe deficient *Sorghum bicolor* (cv. SPV-393).
[b] Not determined.

1982; Vose, 1982). Between species a close positive correlation exists between chlorosis resistance and the release of PS under Fe deficiency (Table 1, Kawai *et al.*, 1988; Marschner *et al.*, 1986). The key role of the PS system for Fe acquisition in graminaceous species has been emphasized elsewhere (Clark *et al.*, 1988; Marschner *et al.*, 1986; Römheld 1987).

There are reports critical of both the effectiveness and specificity of the PS system for field grown graminaceous species (Crowley *et al.*, 1987). Those reports are based exclusively on calculations of stability constants of PS with various heavy metals at concentration ranges of these metals typical for soil solutions in calcareous soils. However, as shown in Table 2 and elsewhere (Awad *et al.*, 1988), PS are also effective in mobilizing Fe from a calcareous soil. On the other hand, PS can mobilize other heavy metals such as Cu, Zn, and Mn from calcareous soils (M Treeby, personal communication; Takagi *et al.*, 1988).

Studies on the uptake rates of the various heavy metal PS chelates by graminaceous species as well as on the effect of other micronutrient deficiencies on PS release are in progress (F Zhang, personal communication).

There is evidence that the two components of the PS system for Fe acquisition are under different genetic control. This evidence is based on the following results:

1) A lower Fe nutritional status is required to induce enhanced PS release than is required to induce enhanced FePS uptake capacity (Römheld, 1987).
2) A different rate of decline in both PS release and FePS uptake capacity with plant age in barley and sorghum (Clark *et al.*, 1988).
3) Inverse relationship between FePS uptake rates and PS release in relation to the chlorosis susceptibility of different plant species (Table 5 and Fig. 2). This aspect is further supported by the high FePS uptake rates of rice (Table 5) and the low PS release found by Takagi (1976) in this highly susceptible species.

Table 6. Comparison of Fe uptake rates in Fe deficient (−Fe) and Fe sufficient (+Fe) cucumber and barley plants supplied with FeEDDHA or Fe phytosiderophores (each 1 μM)

Plant species	Fe nutritional status[a] (preculture +/− 0.1 mM FeEDTA)	Fe uptake rate (nmol g^{-1} root dwt 4 h^{-1}) Fe supplied as	
		^{59}FeEDDHA	^{59}Fe phytosiderophore
Cucumis sativus cv. Chinesische Schlange	+Fe	34 ± 6	33 ± 7
	−Fe	2681 ± 17	2867 ± 21
Hordeum vulgare cv. Europa	+Fe[a]	1.3 ± 0.8	423 ± 26
	−Fe	0.8 ± 0.6	3454 ± 37

[a] Additional daily foliar application of 0.1% (w/v) Fe citrate.

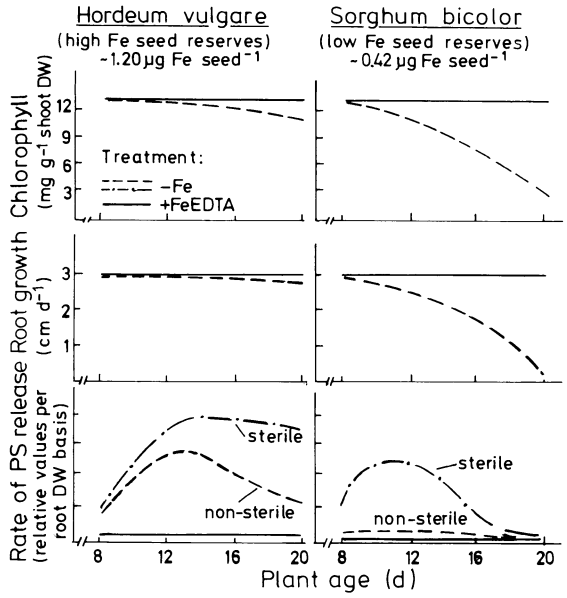

Fig. 2. Effect of Fe supply (+/− 1 m*M* FeEDTA) on time course of chlorophyll concentrations, rate of root growth and release of phytosiderophores by plant species with different Fe seed reserves (*schematic presentation*).

From these results it can be concluded that for adequate Fe acquisition from calcareous soils the rate of PS release rather than the uptake rate of FePS is the relevant factor. Consequently, breeding for high chlorosis resistance in grasses should be focussed on the PS release. Selecting for other characteristics such as Fe deficiency-induced increase in net H^+ efflux (Kannan, 1980) and enhanced Fe(III) reduction at the root surface (McKenzie *et al.*, 1986) would appear to be less promising.

A classification of plant species and genotypes within a species according to the PS release (Table 1) should be interpreted with care. Firstly, besides the distinct diurnal pattern in PS release (Marschner *et al.*, 1986; Takagi *et al.*, 1984) the rate of PS release increases with the degree of Fe deficiency (Kawai *et al.*, 1988; Marschner *et al.*, 1986; Takagi *et al.*, 1984) due to enhanced biosynthesis of PS in the roots (Nishizawa and Mori, 1987). Comparing the PS release in different genotypes at a fixed plant age (*e.g.* Table 1) may therefore be misleading because it ignores, for example, differences in Fe seed reserves. The role of seed reserves is shown schematically in Fig. 2. In substrates with insufficient Fe, higher seed reserves of Fe support, for a longer time, growth of apical root zones, the main sites of PS release (Marschner *et al.*, 1987). Thus, higher seed reserves may delay both the Fe deficiency enhanced PS release as well as the decline in this release due to inhibited root growth under severe Fe deficiency (Fig. 2).

Secondly, microbial degradation of PS during collection of the root exudates can be substantial (Kissel, 1987; Fig. 2). In preliminary experiments, a much higher proportion of the PS was decomposed during root exudate collection under non-sterile conditions in sorghum compared to barley (Fig. 2).

Taking these factors, Fe nutritional status, seed reserves and microbial degradation into account, and given the relatively simple inheritance of Fe efficiency in graminaceous species (McDaniel and Brown, 1982) a successful selection for chlorosis resistance in populations based on release of PS should be possible. Furthermore, increasing the seed Fe content in a given species or cultivar by either selection and breeding or seed pretreatment should also be considered as a method of lowering the susceptibility of Fe deficiency of field grown plants at least during early development. For manganese the success of this approach has recently been demonstrated in wheat (Marcar and Graham, 1986).

Acknowledgements

The authors thank Dr M Treeby for his valuable comments and correction of the English text. Seeds of both barley cultivars Minori-Mugi and Rikuzen-Mugi and cultivar Europa were kindly provided by S Takagi, Iwate Univ., Morioka, Japan and by the breeder H. Hege, Waldenburg, FRG. This work was financially supported by the Deutsche Forschungsgemeinschaft.

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PLSO PN 134

Investigations on the nutrient uptake efficiency of different grape root-stock species and cultivars

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Key words: ammonium nitrate, carbonate compatibility, grapevines, green cuttings, proton extrusion

Abstract

Testing the proton extrusion capacity of roots of different grape rootstocks and cultivars in hydroculture it could be demonstrated that there exists great differences. It could be well demonstrated that differing genetic origin results in a distinct proton extrusion capacity. Ranking the rootstocks by their proton extrusion capacity shows a marked influence of the former parents Berlandieri and Rupestris. Field trials with Riesling grafted on different rootstocks verified also a great influence of them on the mineral accumulation of musts and wines.

Introduction

Grape production throughout the world is carried out on more than 10 million ha and the annual production is about 36 million tons of technical grapes used for wine and juice production. More than 54 million tons of grapes are used as dessert grapes or fresh fruits for direct consumption. Most of the production occurs between the 28th and the 48th to 50th parallels in both hemispheres. It is also found in some tropical areas.

The ecological variability of the genus Vitis is very great, but on the other hand it is necessary to graft all grape-vine varieties on rootstocks resistant to phylloxera. According to this technique it seems that the vigour and nutrient uptake efficiency is influenced. In earlier reports Becker (1960) and Pouget and Juste (1972) observed differing nutrient contents in leaves of grapevine leaves grafted on different rootstocks.

Remarkable differences with respect to nutrient efficiency are described by Saric *et al.* (1982), Marschner and Schropp (1977) and Edelbauer (1979). A genetical predisposition for iron chlorosis of two root-stock varieties was demonstrated by Kolesch (1985).

Proton excretion, *e.g.* production of H^+-ions by the roots and release into the rhizosphere, is a central process in plant nutrition and depends on ATPase localized in the plasma-lemma, which excretes protons in the rooting medium by simultaneous consumption of ATP (Mengel and Steffens 1983; Schubert, 1984). This enzyme produces a shift in the chemical equilibrium in form of a electrochemical membrane potential, which improves the uptake of cations, such as potassium, calcium, magnesia and so on.

Besides the availability of nutrients in the rhizosphere proton excretion has a direct influence on the nutrient uptake. In this connexion the composition of the medium and also the different characters of the plants and cultivars are important on the height of this mechanism.

Because little information exists on these basic processes in grape-vines, investigations were made to test the influence of different rootstock species and cultivars on proton extrusion in hydroculture.

Materials and methods

Species and cultivars

The following species and cultivars listed in Table 1 were used in the investigations.

As can be seen 16 different species were tested: 12

Table 1. Rootstocks, clones and crosses

Rootstock	Cross	Clone
Berlandieri		K1.1 Gm
Riparia 1G		K1.1-47 Gm
Rupestris du Lot		K1.3130 Gm
SO4 Gm	Berl. × Riparia	K1.6-4-22 Gm
5BB	Berl. × Riparia	K1.44-3 Gm
3309 Couderc	Rip. × Rupestris	K1.9-18 Gm
SORI	Solonis × Riparia	K1.92-14 Gm
Börner	Riparia × Cinerea	K1.5135-54 Gm
26 G	Trollinger × Riparia	K1.27-7 Gm
Fercal	BC 1 × 333 EM	
Gm 6495-3	Vitix × *V. amurensis*	
Gm 7116-26	Seibel × *V. vinfera*	
Riesling		K1.198-40
Müller-Thurgau		K1.2

Gm = registered by the Research Station Geisenheim.

rootstock cultivars, 2 Vinifera cultivars, and 2 interspecific hybrids.

Culture methods

All cultivars mentioned above were cultivated in the department of grape breeding of the Research Station Geisenheim. Cuttings were made from suckers by cutting them into 6–8 cm pieces, having one bud. The leaves were reduced to a diameter of 4 cm and therefore transpiration and dissimilation could be efficiently reduced.

The cuttings were sterilized with a 0.05% solution of Chinosol^R (registrated trade mark of 'Hoechst Comp.') and immediately transferred to boxes filled with moist perlite.

The boxes were stored in a glasshouse with an ambient temperature of 30°C and an air humidity of nearly 100%. After 5 days the first rootlets were formed and after further 14 days growth of roots was so strong that it was possible to transfer them into nutrient solutions.

The rooted cuttings were cultivated in a nutrient solution consisting of N, 1.0 mM; P, 0.1 mM; K, 0.5 mM; Mg, 0.3 mM.

The micronutrients were applied as a mixture. The pH was corrected to 7.0 as growth of the roots in the solution preceded and the solution was continuous aerated.

pH-measurements

18 hours before measurements were made the plants were transferred to distilled water. Subsequently they were placed in 250 mL Erlenmeyer flasks filled with 100 mL of nutrient solution (consisting of 1.5 mM $(NH_4)_2SO_4$ and 1.5 mM K_2SO_4). The proton extrusion was measured within 72 h whereby after every 4 hours the pH was determined. All measurements were done at 9.00 am, 1.00 pm, 5.0 pm and 9.00 pm. The measurements were performed with a microelectrode, type U-402-M3 (Ingold). All tests were made in 4 replicates.

Results and discussion

By cultivating the plants within these short experiments in a solution which contains only ammonium and potassium it is possible to get a very clear separation between the different rootstock varieties and cultivars. In another series of experiments it could be shown that compared to a pure nitrate solution or a solution containing 50% ammonium and 50% nitrate the excretion of protons in a pure ammonium solution is very rapid and higher than in the others. This seems logical since in the field the most abundant ion is nitrate. On the other hand it is well known that there exists a relatively good correlation between proton extrusion and calcium carbonate compatibility of grapevine rootstocks.

In Fig. 1 the decrease in pH is shown as an average of all varieties tested.

Two hours after transferring the plants to the solution the pH fell from the neutral point to pH 5; after 12 hours the pH in the solution reached 4. At the end of the test period, 72 hours later, the pH was 3.6. It can be well demonstrated that during the dark phases the decline in pH is not as rapid as during the light phase.

As can be seen in Fig. 2 the amount of excreted protons at the beginning is very low: about 10 μM. The change of pH is from 7 to 5. In the range from 2–12 hours the proton concentration reaches 100 μmoles and after 72 hours the proton concentration is 320 μmoles. It is surprising that in

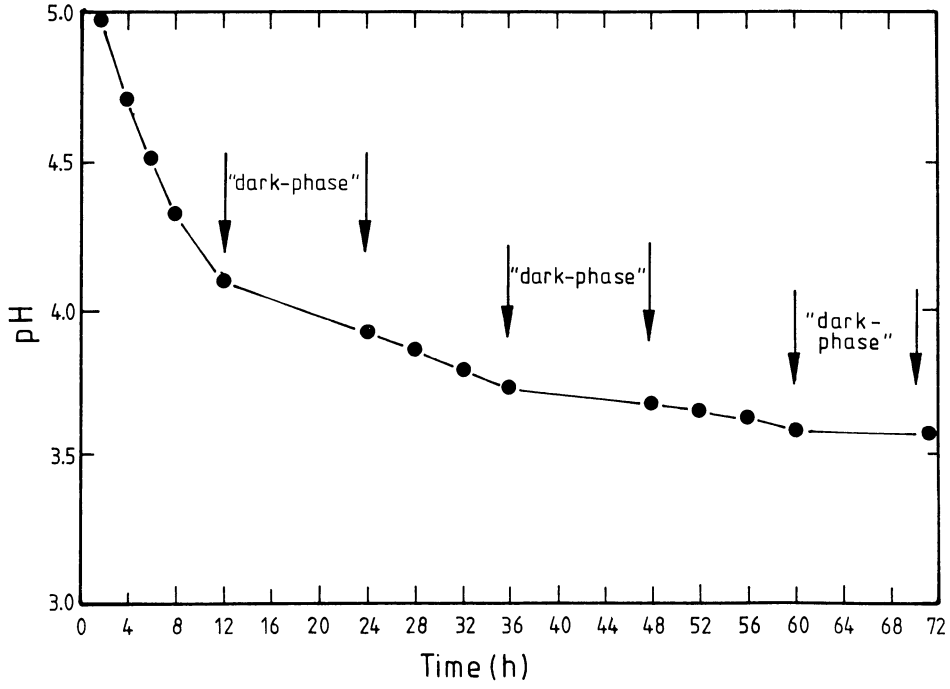

Fig. 1. Changes in pH in an ammonium based nutrient solution by rootstocks. Average of all tested rootstocks.

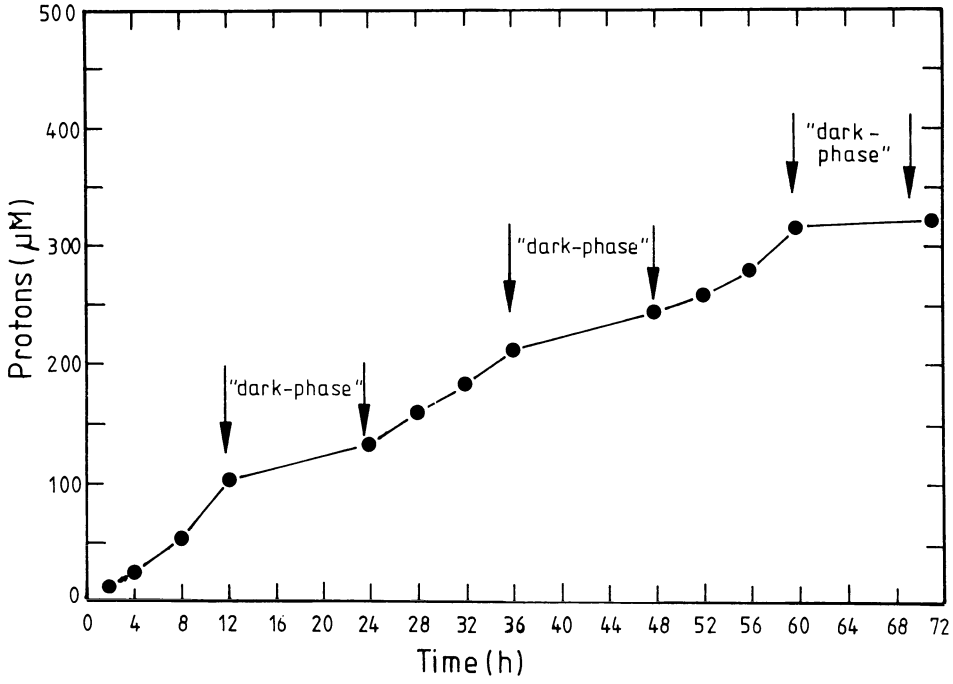

Fig. 2. Changes in proton extrusion in an ammonium based nutrient solution by rootstocks. Average of all tested rootstocks (μmol H^+).

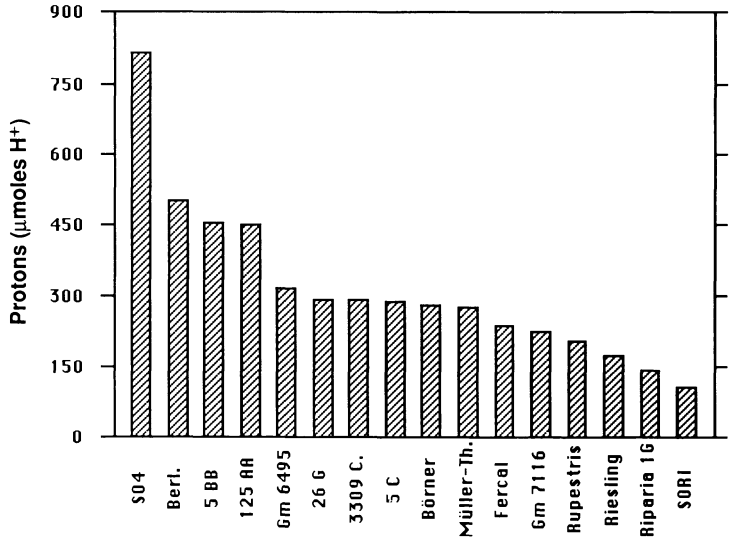

Fig. 3. Proton extrusion by different rootstocks in ammonium based nutrient solution after 60 hours (μmol H^+).

spite of a very high concentration in the bathing solution the plants continuously produce protons. The process seems continuous and is hardly inhibited by the protons in the solution. One can see also a delay during the dark phases *i.e.* the hours 12–24 and 36–48.

Looking at an average gives only a first impression of the possible efficiency of the genus Vitis. But improving plants more information is necessary about special species and/or cultivars.

Testing the above mentioned 16 cultivars in an ammonium based nutrient solution the following results could be obtained (Fig. 3).

After a test period of 60 hours one can see that the species differentiate in a broad range: SO 4 produced 813 μmoles H^+ and SORI 106 μmoles H^+. Recalculated as pH-values the SO4 had 3.10 and SORI 4.07. In this case one can prove, that the use of protons gives more information than pH-values. The Vitis cultivars *Riesling* and *Müller-Thurgau* show a very low proton excretion compared with the most common rootstock SO4. It produces 4.6 times more than *Riesling* and 2.9 times more than *Müller-Thurgau* on their own roots.

Examination of the different rootstock species Berlandieri, Riparia and Rupestris which are used as parents in rootstock breeding, shows that Berlandieri has a relatively high potential of proton extrusion compared with Riparia 1G. The ratio is about 1:0.29. Rupestris is somewhat better and it produces about 41% the protons compared with Berlandieri.

Crosses between Berlandieri × Riparia are in the same range as their parents with exception of SO4, which produces 1.6 times more protons than Berlandieri, probably due to a heterosis effect. On the other hand 5C has nearly the same height as Riparia 1G. SO4, 5BB and 125AA are called 'vigorous' and 5C as 'medium vigorous' rootstocks. These observations made in the viticultural practice are in a good agreement with the proton extrusion capacity of the different rootstocks (Fig. 4).

The cross between (Rupestris × Riparia 1G) 3309 Couderc also has a greater capacity for proton excretion than the parents. It produces twice as many protons as Riparia and 1.4 times that of Rupestris. However in spite of this relative high extrusion capacity 3309 Couderc is not very successful on calcareous soils.

Besides this overall production capacity it is also interesting to know something about proton excretion per unit root fresh weight to compensate for the influence of different root systems (Fig. 5).

The order of rootstocks is the same as in Fig. 3, where the total proton production during 60 hours was demonstrated. It is true that Berlandieri and the two crosses SO4 and 5BB belong to the rootstocks with a considerable efficiency, but 3309 Couderc possesses the highest proton excretion capacity followed by 26G a cross between

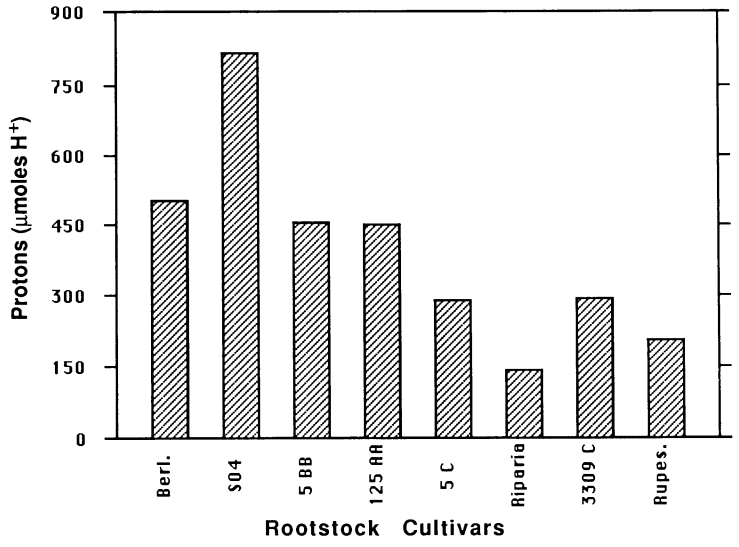

Fig. 4. Proton extrusion by different crosses of Berlandieri, Riparia and Rupestris (μmol H^+).

Vinifera × Riparia. This gives rise to the assumption that the development and distribution of the root system in the soil is a very important phenomenon. But relatively little is known about rooting patterns of grapevine rootstocks. A relatively high amount of protons per gram root fresh weight is also produced by Fercal, a newly developed French rootstock. It is a cross between BC 1 (Berlandieri × Colombard) and 333 EM (Cabernet Sauvignon × Berlandieri). As discussed later the same facts are valid for this cultivar as mentioned for 3309 Couderc.

A possible point for the identification of positive physiological characters of rootstocks is their resistance against chlorosis or their adaption on calcareous soils. This is a very important point

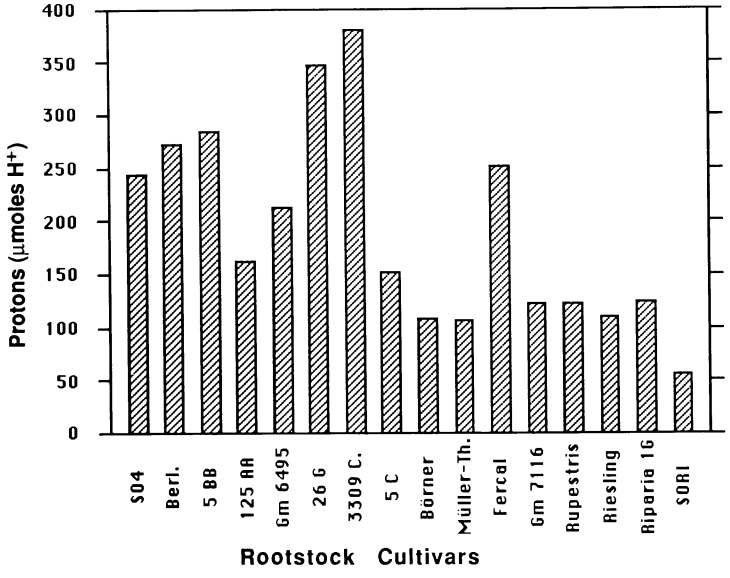

Fig. 5. Proton extrusion of different rootstocks based protons per gram root fresh weight (μmol H^+).

Table 2. Grouping of rootstocks according to their compatibility against calcium carbonate

Rootstock	Good	Medium	Less
SO 4	813		
Belandieri	500		
5BB	455		
125 AA	450		
26 G	293		
5C		291	
3309		291	
Rupestris		206	
Riparia			145
SORI			106
Fercal		236	
Gm 6495	316		
Gm 7116		226	
Bõrner		280	

because most grapevines are planted in soils with calcium carbonate contents ranging from 5–90% $CaCO_3$. Grouping the tested rootstocks after the criteria 'good', 'medium' and 'less' calcium carbonate compatibility (Galet, 1976; Branas, 1974, Pongracz, 1983) it is possible to see a very good agreement between total proton excretion and the degree of calcium carbonate compatibility. The grouping is shown in Table 2.

SO4, 5BB, 125AA and 26G are considered as 'good' compatible; with exception of 26G all rootstocks possess a high rate of proton secretion. In the last case the rooting pattern, which we do not know, can be an explanation for this grouping.

Riparia, in the category of low compatibility also shows a very low capacity for proton extrusion. A less pronounced agreement is shown from 3309 Couderc; in the literature it is grouped in the 'medium' range, but our experiences show that the adaption to calcareous soils is low. As the tests with proton extrusion demonstrate it should be grouped in the last category.

According to the presented results the rootstock cultivar 'Fercal' should be grouped in the category 'medium' and variety SORI in 'less' compatible. In France the first mentioned Fercal is recommended as a calcium carbonate compatible rootstock; experiences in Germany were not convincing. Newer reports about Fercal indicate, that it is very sensitive to a surplus of potassium, developing magnesium deficiency and vice versa, possibly related to the smaller proton production.

These *in vitro* investigations about proton excretion capacity of different rootstock varieties may give some hints of a pronounced genetical influence on nutrient uptake of grapevines.

Table 3. Influence of different locations and rootstocks on the accumulation of nitrogen, phosphorus, potassium, calcium, and magnesium in the must of Riesling (mg L^{-1})

Rootstock	N	P	K	Ca	Mg
Rechacker					
5BB	770*	223	1085	101	77*
5C	665*	226	1010	82	66*
SO 4	697*	230	1000	94	71
125 AA	743*	228	978	106	71
Lorch					
5BB	903	304	1378	119	106*
5C	987	307	1350	99	101
SO 4	910	281	1328	128	89*
125 AA	793	261	1385	109	101
Bechtheim					
5BB	980	92	930	115	92
5C	876	102*	923	74	84
SO 4	863	85*	822	71	83
125 AA	810	86	827	82	84
Hallgarten					
5BB	1130	174	1247*	102	73
5C	1000	152	1190	102	78
SO 4	940	173	1120	119	75
125 AA	1240	175	1115*	106	74

* = significant 5%-level.

Results from a field trial, which began last year, may demonstrate that through this general ability to control qualitatively the nutrient uptake also the composition of the must is influenced.

Testing the most common rootstocks in four different locations one can see, that the crosses from the series Berlandieri × Riparia differ also in their ability to accumulate minerals in the musts. In contrast to the tests *in vitro* the order does not completely agree with results observed in the field trial. As can be seen from Table 3 on 'Rechacker' there is a differentiation between 5BB, 5C and 125AA in N-content and 5BB and 5C in Mg-content. In 'Lorch' 5BB and SO4 can be discriminated in Mg-content; in 'Bechtheim' 5C and SO4 differentiate in P-content, and in 'Hallgarten' 5BB and 125AA differ in K-content.

It is possible that the ability to acquire nutrients from the soil is superposed with the different influences of the location. Analyses made with material from the last vegetation time, where musts from Riparia × Rupestris crosses were enclosed,

show that the minerals in these musts are significantly lower than in the other ones.

Conclusion

Growing rooted green cuttings of different rootstocks in an ammonium based nutrient solution show that

1. rootstocks with differing genetic origin produce different amounts of protons
2. the vigour of root growth has a great influence on the amount of extruded protons
3. the amount of extruded protons referred to a gram root fresh weight leads also to great differentiation between the rootstock cultivars.
4. Berlandieri × Riparia crosses produce the greatest amounts of protons in an ammonium based nutrient solution.

Preliminary field trials demonstrate that the rootstocks have a great influence on the mineral composition of musts and wines. The order of rootstocks found in *in vitro* could not be confirmed by the field experiments and the influence of the location is presumed to have a larger effect.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 93–96.
PLSO PN 135
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Heritability of root characteristics affecting mineral uptake in tall fescue

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Key words: *Festuca arundinacea* Schreb., root diameter, Mg concentration, root volume

Abstract

Modification of plant roots can potentially increase the area of adaptation of tall fescue (*Festuca arundinacea* Schreb.). Limited information is available on the heritability of root diameter and root volume in tall fescue. A greenhouse experiment was conducted with 50 random 'Kentucky 31' (Ky-31) tall fescue parents and their progeny grown in aerated nutrient solution during four separate time intervals. Measurements of root volume, root diameter, shoot Mg concentration, and shoot K/(Mg + Ca) ratio were made six weeks after clonal material was transplanted in nutrient solution. Heritability estimates for root volume, root diameter, shoot Mg concentration, and shoot K/(Mg + Ca) ratios were determined. Broad sense heritabilities were 0.60, 0.65, 0.32, and 0.58 for root volume, root diameter, Mg concentration, and K/(Mg + Ca) ratios, respectively. Narrow sense heritabilities were 0.41, 0.18, 0.19, and 0.58.

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is an important forage crop in the temperate zone of much of the world. Yield potential and stand persistence are reduced, however, due to compaction layers found in many soils, such as the Coastal Plain soils of the southeastern United States. Compaction layer restrict root development and prevent root growth into available moisture and nutrients in the subsoil (Elkins *et al.*, 1977). The potential exists to overcome these inherent limitations and extend the area of adaptation through plant breeding.

The response of tall fescue with differing root morphology to soil compaction and water stress was described by Williams *et al.* (1983). Tall fescue plants with large diameter roots (LDR) were thought to penetrate the compacted layers and access subsoil water. Large diameter roots, compared with small diameter roots (SDR), were associated with increased tolerance to plant parasitic nematodes in the field.

Considerable research has been directed toward selecting tall fescue genotypes that accumulate adequate Mg to prevent hypomagnesemic tetany in cattle (*Bos taurus*). Evidence indicates variation in mineral accumulation in tall fescue is under genetic control and heritability (h^2) is high (Buckner *et al.*, 1981; Nguyen and Sleper, 1981; Sleper *et al.*, 1980). Mineral accumulation, including magnesium uptake, has been related to tall fescue root diameter in studies involving four genotypes (Edwards and Pederson, 1987; Pedersen *et al.*, 1987; Torbert *et al.*, 1985). Genotypes with LDR exhibited lower Mg accumulation than SDR genotypes, indicating that selection for LDR could affect the tetany potential of tall fescue.

In cereal crops, root morphological traits are under genetic control (Hurd, 1976). Differences in rice (*Oryza sativa* L.) root characteristics are related to drought resistance and recovery, and are highly heritable (Ekanyake *et al.*, 1985). Root volume and dry weight in barley (*Hordeum vulgare* L.) 'reflect their capacity to absorb nutrients from well-stirred or flowing solutions' (Hackett, 1969).

Root morphology has an influential role in the persistence and adaptability of tall fescue, and its magnesium tetany potential for cattle. However, neither the inheritance of root morphology traits nor an estimate of their variability within a representative population has been determined. Thus, the objectives of this experiment were: 1) to characterize the variation in root diameter and root volume and to provide heritability estimates for these characters in 'Kentucky 31' (Ky-31) tall fescue, and 2) to describe the relationships between root diameter and volume and shoot Mg concentration of Ky-31 tall fescue.

Materials and methods

Fifty random Ky-31 tall fescue parents and three open pollinated progeny from each parent (free of *Acremonium coenophialum* Morgan-Jones and Gams) were maintained in field nurseries. Ramets of each were brought to the greenhouse and used as a source of material in this greenhouse study. Four similar sized propagules (clonal replicates) were selected from each ramet, all tillers and root tissue were removed, and all leaf tissue except approximately 10 mm of the bottom leaf and its sheath were removed.

The propagules were placed in a 12-L tank containing a nutrient solution with the following composition: 0.25 m*M* KCl, 0.25 m*M* KH_2PO_4, 0.25 m*M* NH_4NO_3, 0.5 m*M* $CaCl_2$, 180 μM FeDTPA (diethylene triamine pentaacetic acid), 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, and 0.05 μM Mo. Magnesium concentration was maintained at 200 μM. Concentrations were maintained by the addition of tap water and nutrients as required. Solution pH was maintained at 5.6 and 5.8 by the addition of HCl or NaOH. All tanks were vigorously aerated and nutrient solutions were changed one to two times during the course of the experiment. Greenhouse temperature ranged from 24 $\pm$ 5°C.

Plants were harvested at six weeks and divided into shoots (leaf blades, leaf sheaths, and stems) and roots. Root volume (mm^3) was determined by measuring the volume of water displaced by the plant root system. Root diameter measurements were made on the three longest primary roots resulting from outgrowth of the propagule. Roots were measured 25 mm behind the root apex using a light microscope and a scaled eyepiece. Roots removed were maintained in a petri dish with moist filter paper and root diameter was determined within 4 h of removal. Shoots were oven dried at 49°C, and stored for Mg concentration determination. Concentrations of Mg, Ca, and K in the shoots were determined by inductively coupled argon plasma spectrophotometry.

Parents and each of their three progeny were grown in separate time intervals (environments) in the greenhouse. In each environment, the experimental design was a randomized complete block with four replications. Means and variances were computed for each trait measured. Analysis of variance was run on the parents and on the progeny grown in three environments. Heritability estimates and their 90% confidence intervals were calculated from the mean squares using the method outlined by Knapp *et al.* (1985).

Results and discussion

Genotype effects were significant for root volume, root diameter, Mg, and the K/(Mg + Ca) ratio in the 50 parents, but were not significant for root volume and the K/(Mg + Ca) ratio in their progeny (Table 1). The genotype × environment interaction was highly significant for progeny root volume, root diameter, and K/(Mg + Ca).

Means, standard deviations, and the range of values observed for these characters in the parents and their progeny are shown in Table 2. Parent root volumes were generally smaller than progeny root volumes, but a considerable range of values was found in both groups. Root diameters and their standard deviations were similar for both groups. Shoot Mg concentration and shoot K/(Mg + Ca) ratio showed evidence of tetany potential in both groups with a considerable range of values in both the parents and their progeny when grown in nutrient solution.

Broad sense heritability estimates (based on parent mean squares) and their 90% confidence intervals are 0.60 ($0.37 < h^2 < 0.70$) for root volume, 0.65 ($0.45 < h^2 < 0.74$) for root diameter, 0.32 ($0.00 < h^2 < 0.74$) for Mg concentration, and 0.58 ($0.35 < h^2 < 0.69$) for shoot K/(Mg + Ca) ratio. Narrow sense heritability

Table 1. Analysis of variance of root volume, root diameter, Mg and K concentrations of fifty random Kentucky 31 tall fescue plants and their open pollinated progeny

	Source	df	Mean square	Prob F > 0
Parents				
Root volume	Rep	3	5.3530	0.0001
	Genotype	49	0.6648	0.0001
	Error	147	0.2666	
Root diameter	Rep	3	0.1046	0.0004
	Genotype	49	0.0461	0.0001
	Error	147	0.0163	
Mg concentration	Rep	3	203654	0.3702
	Genotype	49	285190	0.0393
	Error	147	1933008	
K/(Mg + Ca)	Rep	3	4.6370	0.0001
	Genotype	49	0.4827	0.0001
	Error	147	0.2008	
Progeny				
Root volume	Environment (E)	2	0.225	0.8672
	Rep (E)	9	1.551	0.0001
	Genotype (G)	49	3.283	0.0130
	G × E	98	1.927	0.0001
	Error	440	0.492	
Root diameter	Environment (E)	2	0.433	0.0861
	Rep (E)	9	0.597	0.0009
	Genotype (G)	49	0.073	0.2389
	G × E	98	0.061	0.0001
	Error	440	0.021	0.0001
Mg concentration	Environment (E)	2	3.82×10^8	0.0986
	Rep (E)	9	1.26×10^8	0.2057
	Genotype (G)	49	1.10×10^8	0.2302
	G × E	98	0.92×10^8	0.4984
	Error	440	0.92×10^8	
K/(Mg + Ca)	Environment (E)	2	62.345	0.0001
	Rep (E)	9	1.614	0.0001
	Genotype (G)	49	2.143	0.0283
	G × E	98	1.357	0.0001
	Error	440	0.306	

$^{+}$ $P = 0.05$ F > 1.

estimates (based on progeny mean squares) and their 90% confidence intervals are 0.41 ($0.14 < h^2 < 0.61$) for root volume, 0.18 ($0.00 < h^2 < 0.44$) for root diameter, 0.19 ($0.00 < h^2 < 0.45$) for Mg concentration, and 0.37 ($0.08 < h^2 < 0.58$) for shoot K/(Mg + Ca) ratio. The broad sense h^2 estimates do not account for environmental effects on the phenotype.

The narrow sense h^2 estimates for root diameter and shoot Mg concentration are low and using their 90% confidence intervals they are not different from zero. Selection for these characteristics in nutrient solution is not feasible. Narrow sense h^2 estimates for root volume and the K/(Mg + Ca) ratio are higher. Population means could be shifted for these two characteristics, but because of the magnitude of the h^2 estimates, progeny testing rather than direct phenotypic selection would be indicated.

Table 2. Means and standard deviations of root volume, root diameter, and Mg concentration of fifty random Kentucky 31 plants and their progeny

	Volume (mm^3)	Diameter (mm)	Mg concentration ($mg\ g^{-1}$)	K/(Mg + Ca)
Parents				
Mean	1.51	0.90	1771	4.07
Minimum	0.80	0.71	1168	3.23
Maximum	2.33	1.13	3153	4.85
Standard deviation	0.41	0.11	267	0.35
Progeny				
Mean	2.48	0.85	2674	4.32
Minimum	1.65	0.70	654	3.55
Maximum	4.12	1.03	16990	5.26
Standard deviation	0.52	0.08	3030	0.42

Correlation coefficients (r) for root volume, root diameter, shoot Mg concentration, and shoot K/(Mg + Ca) ratio are shown in Table 3. Significant correlations were found for root diameter and root volume in both parents and their progeny. A positive correlation between root volume and shoot Mg concentration was observed in the progeny.

Selection for large root diameter using nutrient solution in the Kentucky 31 tall fescue population is inefficient. In our earlier studies, genetic differences were observed in root diameter of tall fescue (Pedersen *et al.*, 1987; Torbert *et al.*, 1985), but the

Table 3. Phenotypic correlation coefficients among root diameter, root volume, Mg concentration, and K/(Mg + Ca) for Ky-31 parents and progeny

	Volume	Mg	K/(Mg + Ca)
Parents			
Diameter	0.60*	−0.19	0.12
Volume	—	−0.12	0.30
Mg	—	—	−0.13
Progeny			
Diameter	0.28*	0.22	−0.15
Volume	—	0.34*	0.04
Mg	—	—	0.13

* Prob. R > 0 = 0.05.

results were based on observations of only four genotypes previously selected for very large or small roots. Root volume can be effectively selected; however, the value of increased root volume in tall fescue is not known.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 97–101.
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PLSO PN 137

Root traits of maize seedlings — indicators of nitrogen efficiency?

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Key words: cultivars, nitrogen, roots, tropic climate, *Zea mays* L.

Abstract

Due to the high price of fertilizer the input of N for grain maize production must be kept low in many parts of the world. Low input cultivars have been suggested to meet this requirement. Screening of a group of tropical cultivars revealed two high input, two low input and two intermediate cultivars with regard to N utilization. One of the causes of an interaction between genotype and N fertilization might be differences in root morphology. Screening for such differences at an early seedling stage would facilitate the selection for low input varieties. This hypothesis was tested by growing seedlings of the six varieties at different levels of N until the fourth leaf stage. There was no significant interaction between genotypes and N supply. At low and medium N supply, the total seedling biomass was the same but at low N a higher proportion of dry weight was found in the roots. Total biomass was reduced at high N. Low input and intermediate cultivars had higher shoot and root dry weights than did high input cultivars but no significant differences in root surface area were found. Root surface area was greatest at low N. Number and total length of seminal roots were significantly lower for high input varieties which, in combination with a relatively high root surface area, points to an intensive root type.

Introduction

During the last 30 years, the higher maize yields in the USA were proportional to the increased use of N (Messmer *et al.*, 1984) associated with higher planting density. The authors of this paper propose isolating genotypes which are able to make better use of N and so enhance this relationship. The success of such breeding programs would have the advantage of reducing the costs of fertilizer input and helping to reduce environmental problems linked to high N fertilization. European and Corn Belt material (Chevalier and Schrader, 1977; Di Fonzo *et al.*, 1982; Pollmer *et al.*, 1979) as well as tropical material (Thiraporn *et al.*, 1983; 1987) seem to provide sufficient genetic variability for such goal-oriented selection. Cregan and Van Berkum (1984) suggested using measurements of physiological and biochemical processes as selection criteria for an enhanced N utilization. Some disagreement still exists as to whether root morphology or nitrate reduction limits the rate of nitrate uptake (Veen, 1977). Although tropical and subtropical species reduce a substantial proportion of nitrate in the shoot (Andrews, 1986), a causal explanation of the rate limiting process is not possible because of a correlation between the nitrate uptake of the roots and nitrate reductase activity of the leaves (Cacco *et al*, 1983). According to Robinson (1986), the efficiency of nutrient uptake depends mainly on root length while Pan *et al.* (1985 a; b) found a close relationship between the reduction of nitrate and lateral root proliferation.

Within a set of economically important tropical maize cultivars, considerable genetic variability exists for traits such as grain yield at different N supply (Thiraporn *et al.*, 1983; 1987). The exploitation of this variability could be much improved by

obtaining a better insight into those physiological relationships which make it possible to develop early selection criteria.

Material and methods

The effects of N fertilization on yield and yield components have been described for tropical maize cultivars which were classified according to three different responses to N fertilization (Thiraporn *et al.*, 1987). Two cultivars (low input) yielded comparatively well without additional N and showed only a slight response to N fertilization; two cultivars (high input) required high N fertilization for a satisfactory yield; two cultivars (intermediate) yielded relatively well at low N supply and responded well to additional N fertilization. Seeds of these cultivars were germinated between sheets of wet paper on foam rubber until roots were about 1 cm long. Uniform seedlings were planted in polyethylene tubes (45 cm × 6 cm), which were filled with a mixture of three parts quartz sand and one part loamy soil. Nutrient solution (14% by weight) having the following composition: (mmol L^{-1}): 1 KH_2PO_4, 0.5 K_2SO_4, 2 $MgSO_4$ and 3 $CaCl_2$ was added to the dry substrate. Nitrogen was added to the substrate as NH_4NO_3 in the following amounts (mmol kg^{-1}): 0 (low, N1), (3.75 medium, N2), 13.75 (high, N3). A 14 hour photoperiod gave a light intensity of 80 W m^{-2} at plant height. The average temperature was 26°C. Each plant was harvested at the same physiological age, when the third leaf was fully expanded.

Plants were harvested and measurements were made as follows: dry weights of shoots and roots; number, length and diameter of seminal and adventive roots; length and diameter of two laterals from the upper, middle and lower parts of the seminal and adventive roots. The root surface area was calculated from the number, length and diameter of the seminal, adventive and lateral roots.

An analysis of variance was based on 64 replicates for each fertilization type (32 replicates per cultivar).

Results

Maximum shoot dry weights were found at N2 for all cultivar types. Whereas the values of the extensive and intermediate types were about the same at N1 and N3, the high input cultivars tended to have lower shoot dry weights at N3 (Fig. 1).

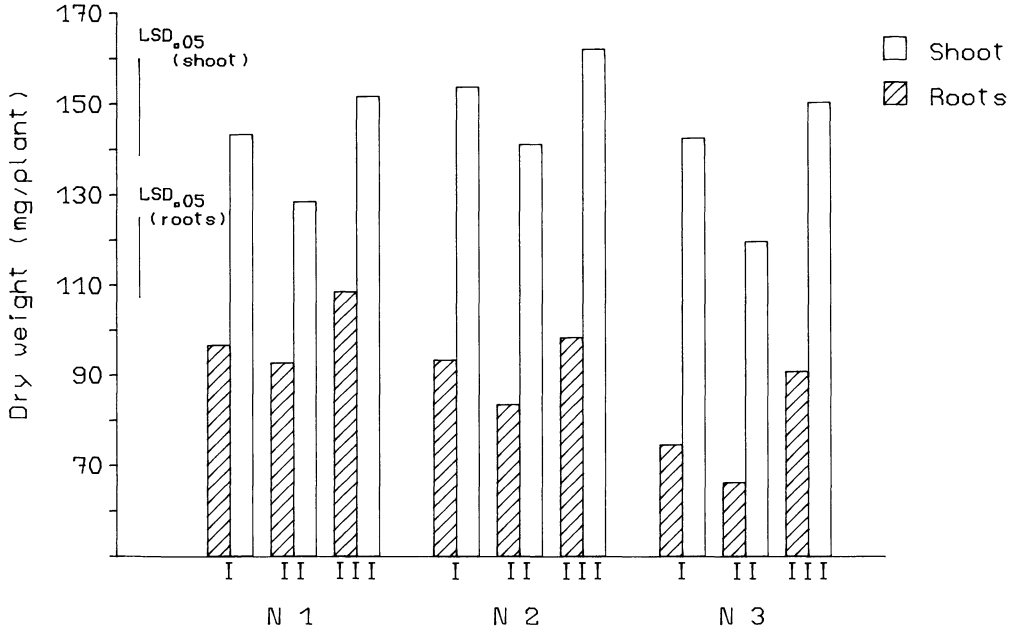

Fig. 1. Seedling shoot and root dry weights of two low input (**I**), two high input (**II**) and two intermediate (**III**) tropical maize cultivars at varying levels of nitrogen. *Average values* are given for each group of cultivars.

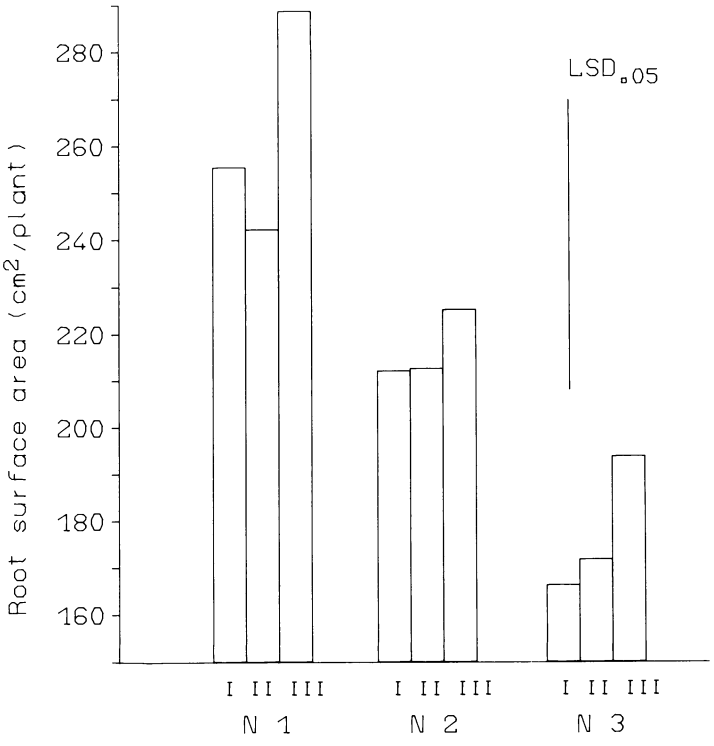

Fig. 2. Root surface area. For further information see Fig. 1.

Although the cultivar types could be described as having rather low values for high input cultivars and high values for the other cultivars, the response to N was quite similar.

Root dry weights were highest at N1 and decreased with a greater supply of N. The most severe declines were found for low and high input cultivars between N2 and N3. Differences between fertilization types were less pronounced than for shoot dry weights. It can be seen from Fig. 1 that the ratio between shoot and root dry weight was lowest at N1 and highest at N3. Ratios differed only slightly between fertilization types. Each cultivar type had the same total seedling biomass at N1 and N2 while at N3 it was about 10% lower.

The cultivar types differed in various aspects of root morphology. The sum of them is represented in the total root surface area (Fig. 2). Differences in root surface area between cultivar types were not significant but the intermediate cultivars tended to have the highest values at all levels of N. As compared to N1, a higher N supply caused decreases in root surface area of 10 to 20% (N2) and 30% (N3). As root dry weights of high input cultivars were comparatively low at all N levels this fertilization type had the highest specific root surface area (unit root surface area per unit root dry weight).

An example of significant structural differences in root morphology is given in Fig. 3. Data are presented as means of N levels because no interaction between N levels and genotypes was observed. The hierarchial grouping of seminal roots by length shows differences between the cultivar types especially from the third root onwards. The medium and higher order seminal roots of the intensive varieties were comparatively short. In this class of roots, the longest roots were formed by the intermediate types. However, the low input varieties developed the longest roots of the very high orders.

Discussion

Genotypes were chosen and grouped according to their utilization of an additional N supply under the conditions of a tropical rainy season (Thiraporn *et al.*, 1987). Varietal differences in response to N fertilization were not reflected clearly by

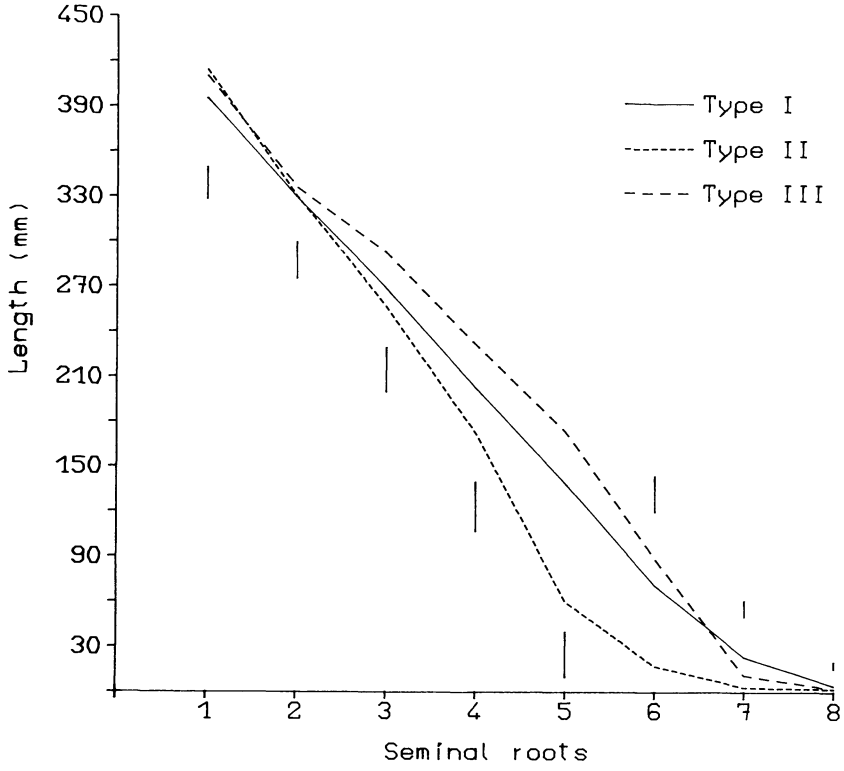

Fig. 3. Length of hierarchically grouped seminal roots, averaged over three levels of N supply. *Vertical bars* represent $LSD_{0.05}$ values. For further information see Fig. 1.

the seedling traits which have been investigated here. This might be due to the criteria by which these groups had been discriminated. Low input and intermediate cultivars were similar with respect to moderately high yields at low N. The main difference between them was a better yield response of intermediate cultivars to a higher N fertilization. The reaction to N fertilization was quite different to that of high input varieties which yielded well at high N only. A comparison of low input and intermediate cultivars with high input cultivars revealed some remarkable differences. High input cultivars were distinguished by a slow accumulation of biomass for the seedlings but the general reaction to N supply was nearly the same for all fertilization types. In accordance with results of Fusseder (1984), the ratio of shoot to root dry weight increased at high N. This was mainly caused by a relatively high portion of root dry matter at low N. A further increase in the shoot/root ratio was found at the highest N level, although this N supply was supraoptimal for shoot growth. Surprisingly, only few differences were observed between fertilization types for root surface area which may be regarded as the sum of all structural elements of the root morphology. The reduction in root surface area due to an increased N supply confirms results of Fusseder (1984) and Geisler and Krützfeldt (1983) that additional N retards root elongation and reduces the number of laterals. Somewhat contradictory results have been reported by Maizlish *et al.* (1980).

High input cultivars used dry matter most efficiently for the development of root surface area but this trait offers no information on the geometry of the root system. The reduced length of higher order primary roots shows that high input cultivars belong to a more intensive root type which exploits a smaller volume of soil more intensively. Similar differences in root types of maize have been described previously (Stamp, 1984).

Tentatively, it can be concluded from these results that some seedling traits may be indicators of the efficiency of N utilization at later stages.

However, investigations must be carried out on larger sets of genotypes in order to describe and to define input types more accurately. As mentioned in the introduction, genetic differences in root morphology cannot fully explain contrasting N utilization of cultivars because other varietal characteristics such as nitrate reductase activity may influence nitrogen uptake as well. However, better acquisition of N from the soil is only one possibility of improving nitrogen economy of genotypes. A more efficient redistribution, perhaps by highly active leaf proteases (Reed *et al.*, 1980), could lead to a superior utilization of already assimilated nitrogen.

Acknowledgements

The authors wish to thank Miss Rungnapa Tangadulratana and Sodsai Changsalag for their valuable assistance. This investigation was supported by the GTZ.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 103–105.
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PLSO PN 138

Varietal differences in root phosphatase activity as related to the utilization of organic phosphates

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Key words: inositol hexaphosphate, nutritional efficiency, organic phosphorus, *Phaseolus vulgaris*, phosphorus uptake, root phosphatases, varietal differences

Abstract

This paper presents results of a comparative study of phosphorus utilization from inositol hexaphosphate by various varieties of *Phaseolus vulgaris* in relation to the activity and characteristics of their root phosphatases. Bean varieties show significant differences in their uptake of phosphorus from inositol hexaphosphate with a clear dependency on the phosphatase activity of their roots at pH 5. The results which are discussed in connection with the phosphorus turnover in the rhizosphere suggest that root phosphatase activity is a significant factor of nutritional efficiency under limited mineral phosphorus supply.

Introduction

A considerable part of soil phosphorus is organically bound. The components of soil organic phosphorus (P_0) fraction however have not yet been fully identified, mainly because of the lack of adequate methods for their extraction and analysis (Dalal 1977). Related to these analytical problems, it has often been difficult to detect changes in soil P_0 during the planting period (Hedley *et al.*, 1982). It is therefore not surprising that the nutritional significance of soil P_0 is generally underestimated. Accordingly, most mathematical models simulating P-uptake by growing plants have been constructed on an entirely inorganic basis (Barber, 1984).

During the last few years, evidence has accumulated which suggests that microbial activity plays a significant role in soil phosphorus transformations (Chauhan *et al.*, 1981). Similarly Helal and Sauerbeck (1984; 1987a) have demonstrated that soil P_0, which is a product of microbial activity is transformed rather rapidly in the plant rhizosphere. In agreement with this, the plant availability of phosphorus from organic phosphates added to soil has been frequently demonstrated. However, the factors controlling the plant availability of soil P_0 are not yet clear. Of special interest is the role of root phosphatases and their genetic dependence.

This paper presents results of a comparative study on phosphorus utilization from inositol hexaphosphate (IP_6) by various bean varieties in relation to the activity and characteristics of their root phosphatases.

Material and methods

In preliminary work, commercially available varieties of *Phaseolus vulgaris* were screened for their phosphatase activity, and the following four varieties covering the whole activity range were selected for the present investigation: Daisy, Erntesicher, Fabiola and Pergousa.

Pregerminated seeds were cultivated in drainable sand culture vessels (2 plants per 1-L pot), and given a full nutrient solution (Helal and Mengel, 1981) for the first two weeks. During the third week a P-free nutrient solution was supplied. This short interruption in P supply was necessary for the

variety-dependent induction of root phosphatases without detectable damage to the plants. At the end of this period one third of the plants (6 replicates) were harvested and analyzed for their P-content and root phosphatase activity (Table 1).

The remaining plants were supplied with the full nutrient solution again, containing 0.2 m*M* P either as KH_2PO_4 (P_i) or as IP_6 for further 2 weeks. At the age of 35 days the plants were cut and analyzed. P-uptake during the last two week period either from P_i or IP_6 was calculated by difference. During the whole experiment the pots were allowed to drain for one hour daily to ensure root aeration and the nutrient solution was renewed every second day.

Root phosphatase activity was determined by the p-nitrophenyl assay adapted from McLachlan and De Marco (1982). For the determination of phosphorus, the plant material was ashed at 490°C, dissolved in 0.1 *N* HCl and its phosphorus content measured photometrically using the phosphomolybdate method as described by John (1970). All the results presented in the tables are mean values of six individual replicates.

Results and discussion

All the varieties tested were able to take up sufficient phosphorus for adequate growth from the initial nutrient solutions. Interruption of the P-supply for one week, did not produce any visible symptoms of P-deficiency. However, as indicated in Table 1, there was considerable varietal differences both in the P-uptake by the young roots and in their phosphatase activity. There is no evidence of a relationship between the P-content of a particular shoot and the weight of its roots. This reflects varietal differences in root efficiency.

Table 1 shows furthermore that root phosphatase activity is pH-dependent. Varietal differences are especially evident in the more acid range (pH 4–5) and diminish at higher pH (6). The root phosphatase activity at pH 5 shows an inverse relation to the P-content of the plant. This dependency of root phosphatase activity on the P-supply of plants has already been discussed by Helal and Sauerbeck (1987b).

As indicated in Table 2, all bean varieties were able to take up phosphorus from the IP_6. However, P-uptake as well as shoot growth was lower in the IP_6-fed plants, except for one variety. The P-uptake in both treatments (P_i and IP_6) showed significant varietal differences. The uptake of IP_6-P ranged from 43 to 98% of the P_i uptake. Referring to the results in Table 1, a positive relation is evident between the utilization of IP_6-P by various varieties and the phosphatase activity of their roots at pH 5. For the variety with the highest root phosphatase activity both P-forms were equally effective as a P-source.

The pronounced pH-dependency of root phosphatase activity (Table 1) is indicative of the significance of the rhizosphere pH not only for the availability of inorganic phosphorus but also for the activity of root enzymes and the related turnover of organically bound plant nutrients. The practical significance of root phosphatase for the nutritional efficiency of plants under field conditions is not yet clear. However, plant roots with high phosphatase activity obviously have the potential to utilize soil organic phosphorus.

It is well known that the plant availability of soil and fertilizer inorganic phosphorus in many areas

Table 1. P-content and root phosphatase activity of 21-days-old plants of various bean varieties grown on nutrient solution. The P-supply was interrupted from day 14 to 21

Variety	Root fresh wt (g plant^{-1})	Shoot dry wt (g plant^{-1})	Root phosphatase activity (μmol plant^{-1} h)			Shoot P-content	
			pH 4	pH 5	pH 6	(mg g^{-1})	(mg plant^{-1})
Fabiola	11.7	1.48	9.3	12.1	4.2	1.82	2.69
Erntesicher	10.1	1.63	7.3	6.9	3.5	2.14	3.49
Pergousa	8.9	1.58	2.7	4.7	3.1	2.30	3.63
Daisy	9.4	1.72	14.5	3.7	3.2	2.64	4.54
$SD_{0.05}$	2.3	0.31	3.7	3.5	1.8	0.42	0.78

Table 2. Phosphorus uptake by 35-d-old plants of various bean (*Phaseolus vulgaris*) varieties. P was supplied in snad-nutrient solution culture (day 21–35) either as KH_2PO_4 (P_i) or as inositol hexa phosphate (IP_6)

Variety	P-supply (0.2 m*M*)	Dry weight (g plant^{-1})	P-content		P-uptake from day 21–35 (mg P plant^{-1})
			(mg g^{-1})	(mg plant^{-1})	
Fabiola	P_i	3.91	3.74	14.62	11.93 (100)[a]
	IP_6	3.72	3.89	14.47	11.78 (98)
Erntesicher	P_i	4.12	3.68	15.16	11.67 (100)
	IP_6	3.81	3.57	13.60	10.11 (87)
Pergousa	P_i	4.32	3.90	16.85	13.22 (100)
	IP_6	3.13	3.51	10.98	7.36 (57)
Daisy	P_i	4.43	4.29	19.0	14.45 (100)
	IP_6	3.28	3.26	10.69	6.15 (43)
$SD_{0.05}$		0.55	0.38	2.80	2.10

[a] Figures in brackets are relative values.

such as the alkaline and calcareous soils in semi-arid regions is low. A way of improving P-utilization from such soils is by the application or organic fertilizers (Elbaruni and Olsen, 1979). Under such conditions the nutritional efficiency of plant varieties with high root phosphatase activity should be investigated.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 107–109.
PLSO PN 139
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The effect of shoot and root genotype on phosphorus concentrations of shoots and roots

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Key words: grafting, morphology, phosphorus content, root effects, rootstock, scion, shoot effects, solution culture, *Trifolium repens*, white clover

Abstract

Genotypes of two morphologically different populations of white clover (*Trifolium repens* L.) were reciprocally and self-grafted. Ungrafted stolon tips were also grown as controls. Grafting *per se* had no significant effect on shoot size, root size, leaflet width or shoot and root % P. The scion genotype had a significant effect on shoot and root size, and leaflet width. Neither scion nor rootstock genotype had a significant effect on either shoot or root % P. However, there was a significant scion × rootstock × P level interaction for shoot % P. This along with other evidence suggests that conflicting results regarding effects of scion and rootstock on % P content of plants within species is probaby due to the interaction of scion and rootstock with environment.

Introduction

Grafting studies have been extensively used to study the effects of scion and rootstock on the mineral nutrition of plants, including the phosphorus (P) content of leaves. However, results have been conflicting, both within and between species. For example, the leaf P content of apples was found to be influenced primarily by scion in some studies (Hanse, 1965; Tukey *et al.*, 1962) but by rootstock in others (Awads and Kenworthy, 1963; Korsal, 1973). The present study used reciprocal grafting as a technique to investigate the effect of shoot and root genotype of white clover upon shoot and root percent (%) P.

Method

Ten genotypes of the cultivar Regal and a semi-natural population (later referred to as population W) were self-grafted and reciprocally-grafted, and the stock rooted in sand. Non-grafted stolon tips were also rooted. Six weeks later, grafted and ungrafted plants that had successfully rooted were transferred to 150 mL pots containing a complete nutrient solution (a modified North Carolina State University solution (Brooking, 1976)) with either 0 or 300 μM P. There were two plants per pot and two replicates, arranged in a randomised block design in a growth cabinet (14 hr day 25°C, 10 hr night 15°C, 200 $\mu E\,m^{-2}\,s^{-1}$). Nutrient solutions were renewed after 10 and 17 days, and plants harvested on day 21. Measurements were made of leaflet width, scion and rootstock dry weight and P content.

Results

Population differences

Ungrafted plants of Regal and population W were morphologically very different. Regal had larger leaves, shoot and root systems, but population W had a higher shoot % P at 300 μM (Table

* This work was undertaken at the Department of Agricultural Botany, University of Reading, Reading, England.

Table 1. The effect of P level on dry weight, leaflet width and shoot % P of ungrafted plants of Regal and population W

Plant character	P level (μM)	Population			$MSD_{0.05}$[a] or $MSR_{0.05}$
		Regal	W	p	
Shoot dry weight (mg)	0	233	69	*	× 1.47
	300	492	84		
	$\bar{x}$	339	76	***	× 1.32
Root dry weight (mg)	0	57	16	*	× 1.84
	300	123	16		
	$\bar{x}$	84	18	**	× 1.54
Leaflet width (mm)	0	9.3	4.9	**	1.0
	300	17.2	7.6		
	$\bar{x}$	13.2	6.2	***	× 0.7
Shoot % P	0	0.24	0.19	*	0.16
	300	0.50	0.79		
	$\bar{x}$	0.37	0.49	*	0.11

[a] MSD, minimum significant difference, MSR, minimum significant ratio.

1). There was no significant difference between the two populations for root % P.

Population W was generally less responsive to added P than was Regal, except for shoot % P (Table 1).

Effect of grafting

Self-grafting had no significant effect on shoot and root dry weight, leaflet width, or shoot and root % P.

Effect of scion and rootstock

There were significant main effects of scion genotype on shoot and root dry weight, and leaflet width (Table 2). Neither scion nor rootstock genotype had a significant effect on shoot % P or root % P. There was, however, a significant ($p < 0.05$) scion × rootstock × P interaction for shoot % P (Table 3); increasing P supply increased shoot % P when Regal was the rootstock, regardless of the scion genotype but, when population W was the rootstock, increasing P supply increased shoot % P when population W was the scion, but reduced it when Regal was the scion.

Table 3. Effect of P level, scion genotype and rootstock genotype on shoot % P

Rootstock / Scion	Regal		W		p	$MSD_{0.05}$[a]
	Regal	W	Regal	W		
0 μMP	0.27	0.24	0.51	0.32	*	0.25
300 μMP	0.76	0.64	0.30	0.60		

[a] MSD, minimum significant difference.

Discussion

The effect of grafting *per se* on shoot and root % P was not significant, although other studies (Eynard and Bukovac, 1962) have shown that the grafting union may restrict P translocation between shoot and root.

Although rootstock had no effect on shoot size (Table 2) in the present study, rootstocks have been shown to determine the size of shoots of many species, *e.g.* apples (Lockard and Schneider, 1981) and grapes (Richards, 1983). In these cases the dwarfing response may be partially the result of a limited supply of an essential nutrient or metabolite from the rootstock (Bukovac *et al.*, 1958).

The higher shoot % P of population W at 300 μM P (Table 1) was not directly affected by either scion or rootstock. Effects of rootstock and scion on shoot % P interacted with each other and with the level of P supply (Table 3). Otsuka (1968)

Table 2. The effects of scion and rootstock genotype upon shoot and root dry weight, and leaflet width. Values given are means of both self and reciprocal grafts

	Scion			Rootstock			$MSD_{0.05}$[a] or $MSR_{0.05}$
	Regal	W	p	Regal	W	p	
Shoot dry weight (mg)	334	156	**	188	278	ns	× 1.57
Root dry weight (mg)	42	22	**	31	30	ns	× 1.79
Leaflet width (mm)	12.5	6.9	***	9.4	10.0	ns	2.4

[a] MSD, minimum significant difference, MSR, minimum significant ratio.

also obtained rootstock × P interactions for shoot % P content of tomato and Datura reciprocally grafted plants. Other studies have shown interactions for shoot % P between scion and rootstock (Axford *et al.*, 1975), and some warn that differences in shoot % P content due to rootstock or scion may vary with year (Hansen, 1965; Tukey *et al.*, 1962) and locality (Korsal, 1973). It would appear that conflicting results in the literature regarding effects of scion and rootstock genotypes on % P content of shoots within species are probably due to the interaction of scion and rootstock with environment.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 111–116.
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PLSO PN 140

Carbohydrate status in roots of two soybean cultivars: A possible parameter to explain different efficiencies concerning phosphate uptake

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Key words: phosphate, root carbohydrate content, soybean, uptake efficiency

Abstract

The carbohydrate levels in the roots of two soybean cultivars (Century, Woodworth) were determined during the early vegetative growth stage. Based on Michaelis-Menten kinetics Century was characterized as being more efficient at phosphate uptake when compared to Woodworth. The experiments were carried out at normal nutrition (+P treatment) as well as under a condition of moderate phosphate stress (−P treatment). The stress situation was created by withdrawal of phosphate from the nutrient solution for five or nine days. Compared to the +P-treatment the deprivation of phosphate led to a reduction of more than 50% in the phosphate content of the plant tissue.

The carbohydrates in the roots exhibited differences between both cultivars with Century generally having higher sucrose levels. During the morning the +P treatment of Century exhibited $\approx 10\,mg\,g^{-1}$ and in the afternoon $\approx 16\,mg\,g^{-1}$ sucrose (plant dry weight basis). This compares to $\approx 8\,mg\,g^{-1}$ sucrose (morning) and $\approx 13\,mg\,g^{-1}$ sucrose (afternoon) for Woodworth. Phosphate stress increased the levels of hexoses (glucose, fructose) in both varieties significantly. The sucrose levels remained higher for the Century variety even during phosphate stress. We conclude that the shoot of the Century variety supplies its root with an increased amount of energy in form of carbohydrates compared to the Woodworth variety. This may enable the plant to take up phosphate more efficiently. It is also remarkable that the Century variety reduces the pH of the nutrient medium more rapidly. This active proton deposition may create a high membrane potential which is known to be necessary for phosphate uptake.

Introduction

The occurrence of variety-specific differences concerning nutrient uptake, nutrient content and nutrient utilization cannot be readily explained by analysis of any single factor. According to Saric (1987) it is a question of morphological and anatomical factors as well as physiological and biochemical processes. In this study the root carbohydrate content was determined for two soybean varieties (Century, Woodworth) which differ in their efficiency of phosphate uptake. This aspect is believed to be of great importance in evaluating different phosphate uptake capacities.

Both the phosphate uptake by the plasmalemma of the root cortex cells and the xylem loading process required carbohydrates as an energy source (*cf.* Loughman, 1987; Sasaki *et al.*, 1984; Ullrich-Eberius *et al.*, 1984). Referring to van Veen (1981) carbohydrates in the root, which are imported from the assimilating shoot, are critical factors involved in processes such as root growth, ion transport, nutrient uptake as well as in the maintenance of the basal metabolic rate. Mechanisms of nutrient uptake contribute 60% of the root respiration (van Veen, 1981). Also, other authors have mentioned the connection between nutrient uptake and carbohydrate status of the root (van Andel *et al.*, 1950; Drew and Saker, 1984; Lee, 1982; Pristupa and Kursanov, 1957). Nevertheless, experiments performed with intact plant systems which include the determination of the carbohydrate content in roots

and comparisons between different genotypes are missing. In this study, soybean at normal nutrition (+P treatment) and in a condition of moderate phosphate stress (−P treatment) were compared. The inclusion of varied phosphate nutritional status made it possible to evaluate the reaction of both soybean varieties under stress conditions.

Materials and methods

Two soybean varieties (*Glycine max* (L) Merr.) which differ in their phosphate capacity were used. The varieties were provided by Prof S A Barber (Purdue University, West Lafayette, Indiana 47907, USA) with the following attributes: characterized by Michaelis-Menten kinetics the Century variety exhibited a value of Imax of $0.74\,pM\,cm^{-2}s^{-1}$, for Km $4.11\,\mu M\,L^{-1}$ and for Cmin $0.20\,\mu M\,L^{-1}$ concerning phosphate uptake capacity. The corresponding values for the Woodworth variety are $0.30\,pM\,cm^{-2}s^{-1}$ for Imax, $5.40\,\mu M\,L^{-1}$ for Km and $0.20\,\mu M\,L^{-1}$ for Cmin. The plants were grown in a nutrient solution basically according to Epstein (1972) with the following nutrient content: macronutrients $1.2\,mM$ KNO_3, $0.8\,mM$ $Ca(NO_3)_2$, $0.1\,mM$ $NH_4H_2PO_4$, $0.1\,mM$ $(NH_4)_2HPO_4$, $0.2\,mM$ $MgSO_4$; micronutrients $10\,\mu M$ KCl, $6.3\,\mu M$ H_3BO_3, $4.0\,\mu M$ FeH_2-EDTA, $0.4\,\mu M$ $ZnSO_4 \cdot 7H_2O$, $0.4\,\mu M$ $MnSO_4 \cdot H_2O$, $0.1\,\mu M$ $Na_2MoO_4 \cdot 2H_2O$, $0.1\,\mu M$ $CuSO_4 \cdot 5H_2O$. During the nursery stage of the plants the nutrient solution was changed weekly. After 25 days the plants reached the experimental stage (vegetative growth stage V4 after Fehr *et al.*, 1971). When ammonium phosphate was withdrawn from the nutrient solution, $0.15\,mM$ $(NH_4)_2SO_4$ was added as nitrogen compensation.

For the determination of the phosphate and carbohydrate contents on plant dry weight basis, the plant material was freeze-dried and ground in a mill (Retsch Company, Type: MM). The phosphate content was determined by the method of Murphy and Riley (1962). The extraction of soluble carbohydrates of the roots was performed with an ethanol/water mixture 80:20 (v:v) after Rathert *et al.* (1981). The carbohydrates were analysed enzymatically (hexokinase method) after the method instructions of the Boehringer Company (West Germany); also the necessary reagents were provided by the Boehringer Company. In all cases the phosphate and carbohydrate concentrations are expressed on the plant dry weight basis ($mg\,g^{-1}$). Mean values and the standard deviation are listed. The Student's t-test was used to identify significant differences between two mean values. (Levels of error of 5%, 1% and 0.1% are shown by the symbols: *, ** and ***.)

Results and discussion

The mean values of the phosphate content in the plant material are listed in Table 1. Referring to data from the literature, $4\,mg\,g^{-1}$ (plant dry weight basis) is the average content of phosphate in plant tissue, therefore the concentration especially of the +P treatment can be classified as high. The average value, however, does not take into consideration different plant parts (*e.g.* shoot, root) nor different ages of the plant tissue. Similarly high phosphate content in the vegetative stage were also found by Borkert and Barber (1983) for soybean, Sawada *et al.* (1983) for dwarf bean, Lee and Ratcliffe (1983) for pea. This is understandable, considering that plants exhibit a high demand for phosphate in the vegetative growth stage and are able to accumulate phosphate in the cell vacuole (Breeze *et al.*, 1984; Edwards and Barber, 1976; Roemer and Schilling, 1986). In comparison to the +P treatment the withdrawal of phosphate for five or nine days greatly reduced the phosphate concentration in the plant tissue. According to experiments of Foyer and Spencer (1986), Lee and Ratcliffe (1983) and Loughman (1987), the reduction of the phosphate content affected principally the inorganic

Table 1. Phosphate content ($mg\,P\,g^{-1}$ dry matter) in plant material in response to varying phosphate nutrition

Treatment/ Variety[a]	# of repl. (n)	Root	Leaves
+P/C	7	21.1 ± 1.6 ***	11.1 ± 1.1 ***
+P/W	5	17.6 ± 1.1	8.9 ± 0.8
−P/C 5d	7	6.1 ± 1.1	4.9 ± 0.7
−P/W 5d	5	5.9 ± 1.3	4.8 ± 0.7
−P/C 9d	4	3.7 ± 0.7	3.6 ± 0.4
−P/W 9d	4	3.0 ± 0.4	3.4 ± 0.3

[a] Variety Century (C) and Woodworth (W); +P = normal nutrition; −P = withdrawal of phosphate for 5 days or 9 days.

phosphate content of the vacuole. The cytoplasmic phosphate content is in general only slightly influenced by different phosphate levels. This is supported indirectly by the fact that the withdrawal of phosphate from the nutrient solution did not affect growth of the experimental plants (Burauel, 1987). A significant, variety-specific difference in the phosphate content was only present in the +P treatment (Table 1). The lower concentration of the Woodworth variety is probably due to its poorer efficiency in phosphate uptake compared to the Century variety.

Figures 1, 2 and 3 show the carbohydrate content of the soybean roots. Regardless of the phosphate supply the root sucrose content of the Century variety was $\approx 10\,mg\,g^{-1}$ in the morning and $\approx 16\,mg\,g^{-1}$ in the afternoon (Fig. 1). Similar values for sucrose concentrations in soybean roots were reported by Kerr *et al.* (1985). The glucose and fructose concentrations were less than $2\,mg\,g^{-1}$ independent of the time of day; the hexose content, however, was significantly increased with the −P treatment (Fig. 1). Generally, the Woodworth variety had a lower root carbohydrate content (Fig. 2). In all cases the sucrose level was significantly (***) lower in comparison to the Century variety. The −P treatment generated a significant increase in the hexose concentration also in case of the Woodworth variety. Furthermore, the Woodworth variety exhibited a significantly reduced sucrose level in the afternoon with the −P treatment compared to the +P treatment (Fig. 2).

When the phosphate withdrawal was extended to nine days increased carbohydrate levels were detected (Fig. 3). Again, predominantly, the hexose content was increased in comparison to the corresponding +P treatment. The significant differences in sucrose content between the varieties remained evident. In addition, the fructose levels were significantly higher in roots of the Century variety in this experiment. According to Burauel (1987) the moderate phosphate stress created in those soybean plants enhanced the assimilate import to the roots compared to plants at a normal nutritional level. Possibly the higher carbohydrate content is contributing to the well known fact that plants exhibit an increased phosphate uptake rate under conditions of phosphate stress (Clarkson and Scattergood, 1982; Drew and Saker, 1984; Katz *et al.*, 1986; Lee, 1982). As mentioned before, the varieties differ in their phosphate uptake capacity. The Century variety exhibits a 60% higher Imax

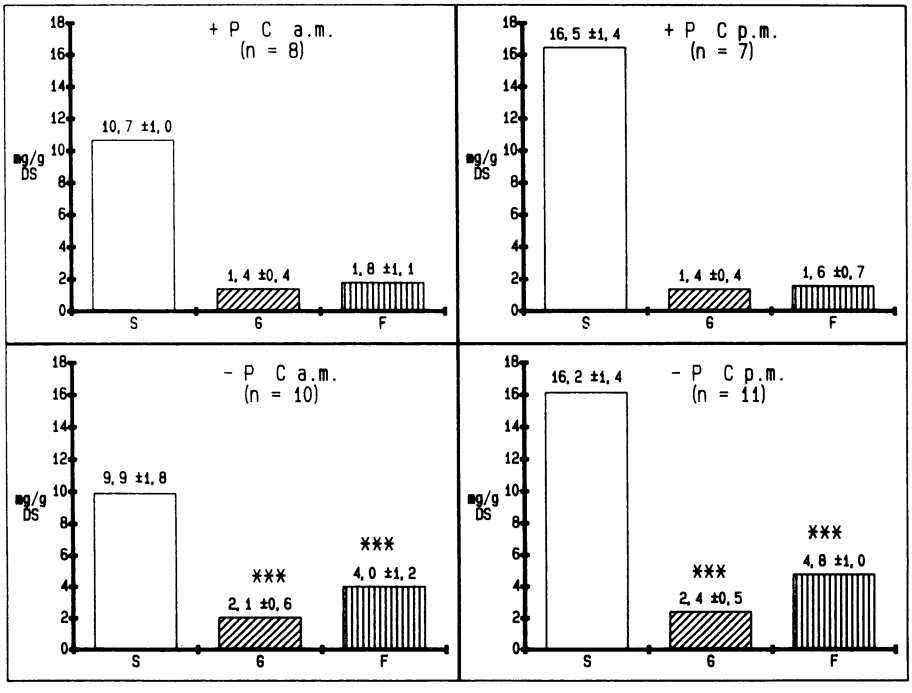

Fig. 1. Carbohydrate content in the root of the Century (**C**) variety in response to phosphate nutritional status and time of day at stage V4. +P = normal nutrition; −P = 5 days withdrawal of phosphate; S = sucrose, G = glucose, F = fructose.

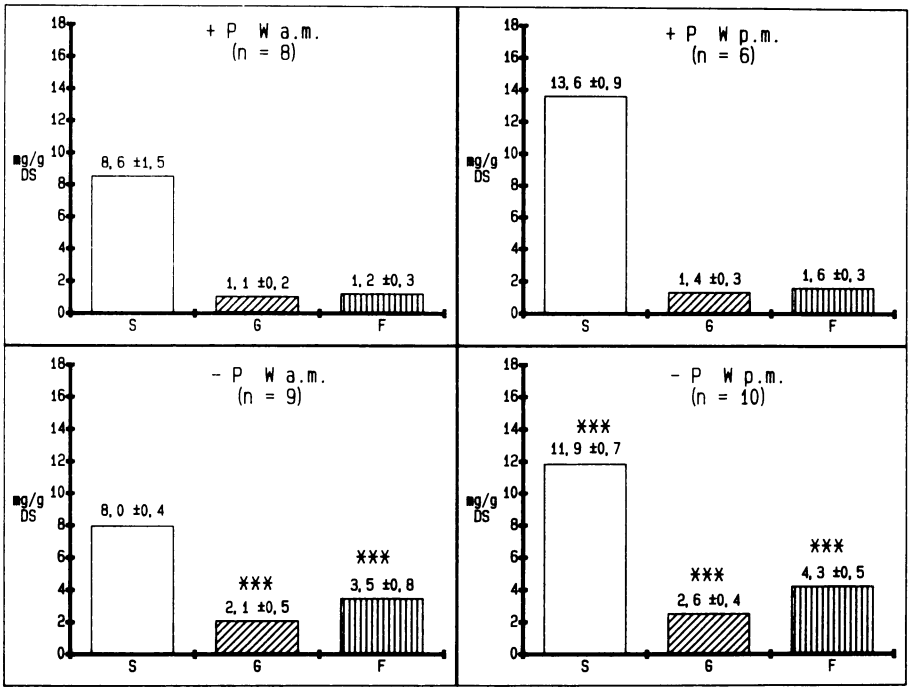

Fig. 2. Carbohydrate content in the root of the Woodworth (**W**) variety in response to phosphate nutritional status and time of day at stage V4. +P = normal nutrition; −P = 5 days withdrawal of phosphate; S = sucrose, G = glucose, F = fructose.

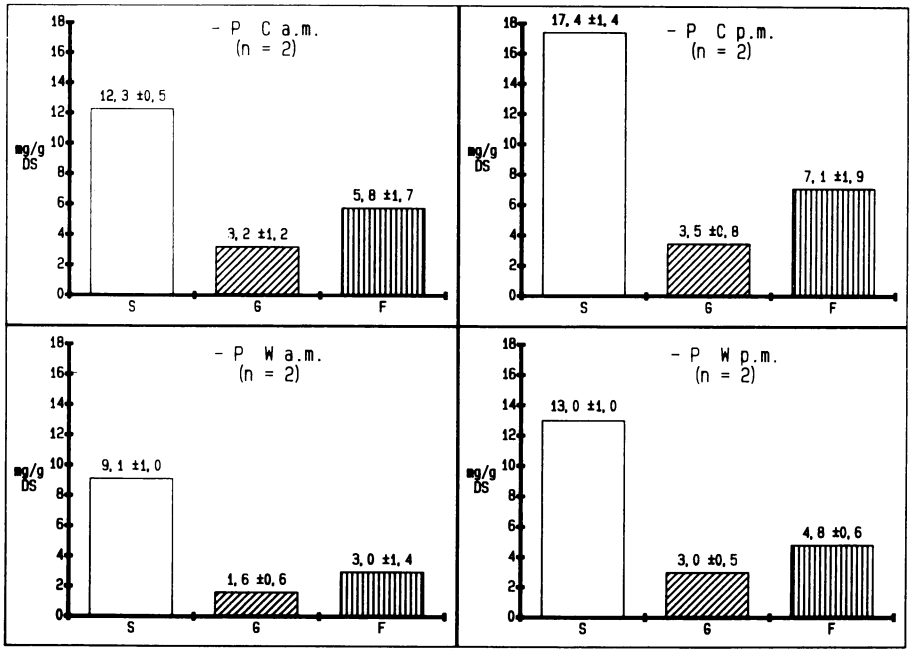

Fig. 3. Carbohydrate content in the root of the Century (**C**) and the Woodworth (**W**) varieties in response to phosphate nutritional status and time of day at stage V4. −P = 9 days withdrawal of phosphate; S = sucrose, G = glucose, F = fructose.

value and a 24% smaller Km value compared to the Woodworth variety. According to Cogliatti and Clarkson (1983) and Lee (1982) a higher Imax value implies an increased number of carriers per unit root and a smaller Km value describes a better affinity of the carrier for phosphate. Differences in these parameters probably require a varied supply of carbohydrates either as structural material or as an energy source.

The change in the pH of the nutrient solution in response to the phosphate supply is illustrated in Fig. 4. The Century variety decreased the pH value more intensively compared to the Woodworth variety with all treatments. With a starting value of pH 6.70 of the +P nutrient solution the Century variety generated a value of pH 4.94 and the Woodworth variety of pH 5.68 within an elapsed time of five days. The −P treatment resulted in values of pH 4.15 for the Century variety and 4.31 for the Woodworth variety with an initial pH value of 5.50 of the nutrient solution. The root mass of both varieties did not display any significant difference which might have had an effect on the pH values.

The decrease of pH values in nutrient solution is promoted by proton extrusion from the roots (Poole, 1978). Because of this active proton deposition an electrochemical potential at the plasmalemma membrane is established, which enables both the passive uptake of cations along the gradient and the phosphate uptake by a H^+/Co-transport mechanism (Poole, 1978; Schubert and Mengel, 1986; Ullrich-Eberius *et al.*, 1984). Roemheld *et al.* (1984) described energy-consuming ATP (adenosintriphosphate)-dependent proton pumps which could be totally suppressed by addition of an ATPase inhibitor. Consequently the supply of carbohydrates as an energy source for the proton pumps is required to establish a membrane potential necessary for phosphate uptake (Ullrich-Eberius *et al.*, 1984). Furthermore, carbohydrates can be used as structural material to maintain intact membrane systems, which can prevent a passive influx of hydrogen ions (Schubert and Mengel, 1986) influencing the membrane potential negatively. We conclude that the shoot of the Century variety compared to the Woodworth variety has supplied its root with an increased amount of carbohydrates under normal nutrition as well as under conditions of phosphate stress. This may enable the variety to take up phosphate more efficiently by using the carbohydrates either as structural material or energy source for the factors related to phosphate uptake.

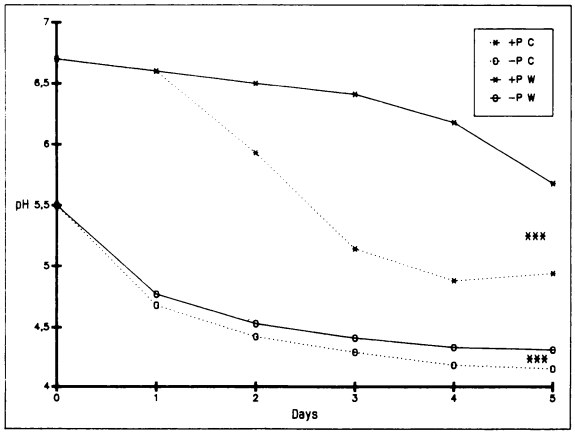

Fig. 4. The change of pH values in nutrient solutions in response to phosphate nutrition. **C** = Century, **W** = Woodworth; +P = normal nutrition; −P = withdrawal of phosphate.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 117–121.
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PLSO PN 141

Properties of potassium uptake by seedling roots of grape cultivars*

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Key words: grape nutrition, H^+ efflux, K^+ leakage, K-ATPase, potassium uptake

Abstract

Uptake rates of (^{86}Rb)K^+ by seedling roots of six cultivars were measured and compared with K^+ content of the root, K^+ leakage, H^+ efflux, and K^+-ATPase activity of a partially purified plasmalemma fraction.

Different cultivars showed significantly different rates of (^{86}Rb)K^+ uptake. The uptake rates of the first (0–5 min) period did not correlate with K^+ content of the seedling roots.

The rates of uptake in the 10 to 30 min period, supposed to be active, were negatively correlated with K^+ content of the root. Roots consistently leaked K^+ during the first 5 min. This leakage was positively correlated with the endogenous K^+ content of the tissue.

H^+ efflux was significantly different among the cultivars and correlated with the K^+-ATPase activity of a microsomal fraction partially purified on discontinuous (18/34%) sucrose gradient. The relationships among transport parameters are discussed.

Introduction

Short term experiments of K^+ uptake by grape roots, intended to test plasma membrane properties, showed that (^{86}Rb)K^+ influx as a function of time was not linear. The uptake rate from 0 to 5 min was two to three times higher than the rate from 10 to 30 min (Maggioni and Varanini, 1983; Varanini and Maggioni, 1982). Furthermore, it was shown that the rate in the first period was scarcely affected by low temperature and dinitrophenol (2,4-DNP) and inhibited by treating roots with Ca^{2+}, whereas the uptake rate in the subsequent period (10–30 min) was much more sensitive to low temperature and 2,4-DNP and stimulated by Ca^{2+} (manuscript in preparation).

A comparative study on roots of different grape cultivars was carried out to evaluate the variability of different parameters able to affect K^+ uptake, such as the K^+ content of grape seeds and seedling roots, and the time-course and rate of K^+ and H^+ leakage. Moreover, (K, Mg)ATPase activity of the microsomal fraction of different cultivars was determined and the relationships among all measured parameters were studied.

Materials and methods

Seeds of six grape (*Vitis vinifera* L.) cultivars (Raboso, Merlot, Cabernet, Moscato, Verduzzo and Marzemino) were collected, accurately washed and surface sterilized before drying in air stream at room temperature. Merlot and Moscato seeds were sampled from two different areas of Veneto region in order to evaluate the influence of different environments. Seedlings were obtained by the method already reported (Varanini *et al.*, 1988). K^+ analysis of seeds and roots were carried out as previously described (Maggioni and Varanini, 1983). K^+ uptake was measured by using ^{86}Rb labelled KCl solutions, according to the already described procedure (Maggioni and Varanini, 1983). Proton leakage was measured by a Radiometer 26 pH meter equipped with a combined glass electrode in

* Part of the present research was carried out at the Department of Agricultural Biotechnology of University of Padova, Italy.

10 mL of distilled water, previously adjusted at pH 6.1 with 10 m*M* KOH, bathing root apparatus (about 200 mg fresh weight), at 28°C, with continuous stirring. ATPase activity was measured on a partially purified plasma membrane fraction isolated by means of a discontinuous (18/34%) sucrose gradient from root tissue according to the procedures already reported (Varanini *et al.*, 1988).

Results

Seeds of six grape cultivars were washed and treated as for inducing germination, then analyzed for K^+ content. Table 1 shows quite uniform values, between 64.8 and 86.3 μmol g^{-1} DW, with only one sample (Merlot 2) significantly lower (48.1 μmol g^{-1} DW). When we compare this single figure with the Merlot 1 sample, different as to geographical origin, we can deduce that seeds of the same cultivar but different origin can be different as far as K^+ content is concerned. In contrast the two samples of Moscato showed K^+ contents not significantly different. Therefore, total K^+ content of grape seeds seems to be quite variable and not dependent on genetic characteristics. Seedling roots of these cultivars, were significantly different in total K^+, from 16.8 to 30.4 μmol/g^{-1} FW (Table 1). Total root K^+ was not correlated with total seed K^+. However, roots of Merlot 1 and Merlot 2 contained K^+ at not significantly different levels, and the same results was obtained with roots of Moscato 1 and Moscato 2. This suggests that during development root apparatus can control its own content of K^+, irrespective of total seed K^+.

Roots of different grape cultivars took up (^{86}Rb)K^+ from a 0.5 m*M* KCl solution at rates ranging from 240 to 420 nmol g^{-1} FW h^{-1} within the first 5 min, whereas in the 10 to 30 min period uptake rates were 102 to 219 nmol g^{-1} FW h^{-1} (Fig. 1). Differences between cultivars were significant both in the first and in the second uptake period. Ratios between the uptake rate in the first (V_1) and in the second period (V_2) were calculated and they resulted also significantly different, showing that the behaviour of cultivars was very variable with regard to uptake rates and rate changes passing from the first to the second uptake period. Nevertheless, it is noteworthy that roots obtained from seeds of the same cultivar but different origin behaved exactly in the same way, without significant differences as far as rates and rate changes are concerned. Uptake rates of the first period were not correlated with total K^+ content of roots, whereas the uptake rates of the second period were negatively correlated ($r = -0.78$, $p \leqslant 0.05$) with total K^+ of roots.

Table 1. Potassium content of seeds and seedling roots of grape cultivars. Letters indicate groups of means that do not differ at the 5% level by Duncan's test

Cultivar	Seeds (μmol K^+ g^{-1} dw)		Roots (μmol K^+ g^{-1} fw)	
	$\bar{x}$	± SD	$\bar{x}$	± SD
Cabernet	86.3a	2.5	24.1abc	4.0
Verduzzo	84.3a	5.2	22.6bc	2.6
Raboso	82.3a	9.8	30.4a	0.4
Merlot 1	78.6ab	4.2	26.1ab	0.4
Marzemino	71.9ab	9.2	24.0bc	4.4
Moscato 1	70.9ab	8.2	16.8c	0.4
Moscato 2	64.8b	14.2	20.0bc	3.3
Merlot 2	48.1c	1.9	23.7bc	1.6

Grape roots submerged into the buffer solution lose 6–13 μmol K^+ g^{-1} FW, corresponding to 25 to 30% of total potassium (Table 2). Efflux rate was very high (67–136 μmol g^{-1} FW h^{-1}) within the first 5 min and very low (0–4.8 μmol g^{-1} FW h^{-1}) in the subsequent period. It was significantly different within the four grape cultivars tested and postively correlated with potassium content of roots.

H^+ leakage was also significantly different as a function of cultivars. Table 3 shows that it increased up to 10–30 min, then decreased until 60 min. After changing the solution in contact with roots, H^+ leakage started again. However, the rate was much lower than the initial, though proportional to the latter. H^+ leakage was also correlated ($r = 0.96$, $P \leqslant 0.05$) with K^+ leakage.

Table 4 shows that K-stimulated ATPase, which is supposed to be representative of plasma membrane ATPase activity, was significantly higher for Cabernet microsomes in comparison with Verduzzo, Moscato and Raboso, which were at the same level. Total activity of roots was also at the highest level for Cabernet, with Verduzzo and Moscato at intermediate values and Raboso roots showing quite scarce ATPase total activity. Among the relationships tested, the only one which proved to be significant was the correlation between K-ATPase specific activity and H^+ leakage from roots ($r = 0.95$, $P \leqslant 0.05$).

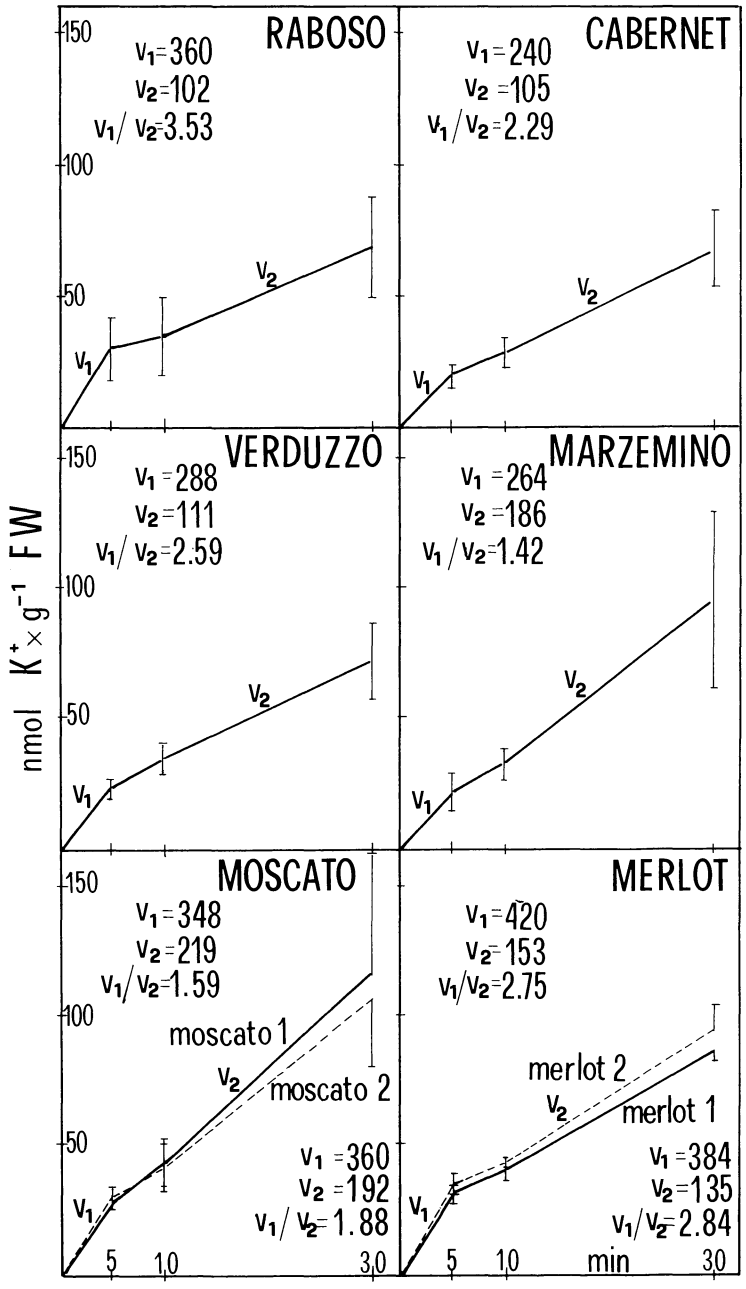

Fig. 1. Time-course of (^{86}Rb)K^+ uptake by seedling roots of grape cultivars.

Discussion

Experiments presented here show that the lack of linearity of (^{86}Rb)K^+ uptake as a function of time (0–30 min interval) is a general feature of grape root tissue. In fact, all the cultivars tested showed uptake rate in the first 5 min higher than in the subsequent 10–30 min period. However, significant differences were observed among cultivars both in the first and in the second period. Ratios between uptake rates in the first and in the second period were also significantly different. Reproducible results obtained with seedling roots of the same cultivar but of different origin indicate that this

Table 2. Potassium efflux from grape roots as evaluated by the potassium content of roots after treatment in 1 m*M* Tris-Mes pH 6.5 buffer solution. Figures represent means ± SD

Cultivar	K^+ content of root tissue (μmol g^{-1} fw)			
	0 min	5 min	10 min	30 min
Raboso	30.6 ± 4.0	20.0 ± 0.3	20.6 ± 0.4	19.7 ± 2.5
Cabernet	26.9 ± 2.9	15.5 ± 0.9	15.8 ± 1.5	13.5 ± 1.9
Verduzzo	24.4 ± 3.3	18.2 ± 1.2	19.7 ± 1.8	18.3 ± 0.9
Moscato	22.3 ± 0.4	16.7 ± 1.6	16.0 ± 0.6	15.7 ± 1.5

behaviour of roots can be genetically based. K^+ efflux rates in the same periods indicate a massive passive discharge of potassium from tissue free-space and H^+ efflux takes place mostly in the first 5 min. Therefore, the (^{86}Rb)K^+ uptake of the first 5 min period seems to reflect an apparent and passive movement through the plasmalemma (Maggioni and Varanini, 1983) or non reversible binding in the free space (Jensén, 1981). On the other hand, it is unaffected by treatments able to inhibit metabolism. Uptake rate in the second period appears to be negatively correlated with the K^+ content of the roots, suggesting a regulatory function of internal potassium, as already suggested (Glass, 1976). Therefore, cultivars may differ with respect to both free space properties (Maggioni and Varanini, 1983) and transport at the level of plasmalemma. Changes of plasma membrane permeability, as observed in maize roots (Pinton *et al.*, 1987), seem not to be involved, since K^+ and H^+ efflux from roots was very high only for a few minutes, with a time course typical of free-space unloading.

Correlation between K-stimulated ATPase activity and (^{86}Rb)K^+ uptake, as reported for cereal roots (Hodges, 1973), was not observed. This could be due to differences in the efficiency of coupling between energy source and membrane carriers (Wignarajah *et al.*, 1983). On the other hand, the role of plasma membrane ATPase in K^+ transport is not clearly understood (Briskin, 1986). The positive correlation observed between K-stimulated ATPase and H^+ efflux from the roots supports the view that H^+ transport across the plasma membrane is the primary active transport process of root cells, to which ion transport may be linked (Sze, 1985). Furthermore, it was also observed that in grape plants H^+ excretion may be independent of ion uptake (Mengel and Malissiovas, 1982). The different transport properties observed among grape cultivars may be related to the capacity of

Table 3. H^+ leakage from seedling roots of grape cultivars, evaluated from pH measurements of distilled water bathing root apparatus. For details see Materials and methods. A representative experiment is reported. (*) indicates change of the solution

Cultivar	H^+ efflux (nmol g^{-1} fw) at min								
	5	10	30	60	*	65	70	90	120
Raboso	664	730	478	372	*	25.9	54.1	60.7	73.0
Cabernet	902	1022	1110	1064	*	65.0	145.2	259.3	410.4
Moscato	484	584	533	403	*	31.9	47.6	49.6	77.8
Verduzzo	310	338	250	169	*	15.4	18.8	15.1	10.1

Table 4. Mg-dependent and K-stimulated ATPase activities of partially purified plasma membrane fractions isolated from seedling roots of grape cultivars. Figures (means ± SD) represent the increase of activity caused by the addition of each ion

Additions	Verduzzo	Moscato	Raboso	Cabernet
	Specific activity (μmol *Pi* mg^{-1} *prot* h^{-1})			
None	3.50 ± 0.57	2.60 ± 0.42	4.15 ± 0.49	3.30 ± 0.57
3 m*M* Mg	12.40 ± 3.54	9.00 ± 1.84	7.10 ± 1.27	8.80 ± 3.11
50 m*M* K	7.40 ± 0.57	8.70 ± 2.12	7.05 ± 1.20	12.40 ± 0.99
	Total activity (*nmol Pi* h^{-1} g^{-1} *of roots*)			
None	40 ± 10	30 ± 10	20 ± 10	40 ± 10
3 m*M* Mg	160 ± 60	90 ± 40	40 ± 10	100 ± 30
50 m*M* K	90 ± 10	80 ± 10	40 ± 10	140 ± 10

adaptation to different environments (Mengel and Malissiovas, 1982).

Acknowledgement

This work was supported by Consiglio Nazionale delle Ricerche, Rome – Italy.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 123–125.
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Sterol content and efficiency of ion uptake by roots of maize genotypes

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Key words: maize, sterols, sulphate uptake

Abstract

Data of compartmental analysis of sulphate were compared with the sterol content of roots of differently yielding maize genotypes. In conditions of steady state nutrient supply, sterol content was significantly correlated only with sulphate efflux (ϕco). This increased at increasing concentration of sterols in the roots. Influx to cytoplasm (ϕoc) was evaluated after sulphate deprivation leading to an induced rate of sulphate uptake. This was negatively correlated with sterol content, which was lower in the high than in the low yielding genotypes. When the highest yield genotype was grown at different sulphate concentrations, influx, efflux, root content of sulphate and sterols were positively correlated with the concentration of sulphate in the nutrient medium. Sterol content in roots appears to be controlled by both the genetic settlement and the nutritional status in maize. Low sterol content is connected with a high efficiency of sulphate utilization.

Introduction

There is increasing evidence that membrane efficiency may be considered among the physiological determinants of crop growth and productivity (Charles-Edward, 1982).

The fluidity of the lipid-protein network of membranes has been shown to play an important role in the control of senescence (Matile, 1984) and in the adaptation to environmental stresses critical for crop yield (Blum and Eberson, 1981; Havaux *et al.*, 1984). An optimum of fluidity can be obtained by modulating the protein to lipid ratio and the lipid composition.

In particular sterol content can affect the permeability of artificial bilayer membranes (Benz and Cros, 1978). In plant membranes the sterol level is controlled by genetic and environmental factors. It is therefore a candidate for the control of the efficiency of nutrient uptake by roots with regard to both the transport mechanism and the adaptation to adverse environment conditions (Kuiper and Kuiper, 1978).

Previous experiments (Saccomani and Ferrari, 1986) showed that grain yields of maize genotypes were correlated with data of influx and efflux of sulphate, evidencing the capacity of roots to control nutrient leakage in conditions of steady state supply and to restore the sulphate inner concentration after the depletion following a period of deprivation.

In the present work four differently yielding maize genotypes were used in experiments of sulphate uptake either in conditions of steady state nutrient supply or after sulphate deprivation.

The same genotypes were used to control the sterol content of roots and to evaluate its relationship with the grain yield and with the influx-efflux of sulphate at different sulphate concentration in the nutrient medium.

Materials and methods

The two maize inbreds A and B were derivatives of the B37 and A59 lines supplied by the De Kalb Center, Chiarano, Italy. Germination of seeds and preparation of seedlings were carried out as previously described (Saccomani *et al.*, 1984). Sulphate influx was determined in 9-day-old seedlings grown in 1:10 diluted Hoagland and Arnon (1950) solution. Sulphate depletion was obtained by replacing sulphate with chloride.

At the beginning of each uptake experiment radioactive labelling was performed with carrier free $^{35}SO_4^{2-}$ and radioactivity in the root homogenate was measured by liquid scintillation spectrometry.

Sulphate efflux was determined by evaluating the radioactivity washed out by non-labelled medium from seedlings previously loaded for 72 h with ^{35}S sulphate. Sterol content was determined by the Lichbermann-Burchardt method (Lynch *et al.*, 1963).

Results and discussion

The sterol content of maize roots was higher in the low than in the high yield genotypes, irrespectively of the nutrient conditions of roots (Table 1). Therefore sterol content appears negatively correlated with the level of heterosis and with productivity. However the nutrient status was not without effect on the sterol level. This was significantly lower in the hybrids after a period of sulphate deprivation. Since the deprivation results in a high level of stimulation of the uptake capacity (induced rate of transport) the efficiency of sulphate uptake appears to be connected with a low level of sterols. The above behaviour was better shown by the data of sulphate uptake and sterol content obtained with the high yielding B × A hybrid in complete nutrient solution after different levels of sulphate deprivation (Table 2). When the level of deprivation increased and, as a consequence, the uptake capacity was stimulated, sterol content decreased. At increasing level of sterols sulphate efflux increased in accordance with a higher fluidity of the membranes, affecting possibly the efflux to influx ratio and by consequence the net uptake.

Table 1. Sterol content of roots of two inbreds (A, B) and two derived hybrids (A × B, B × A) of maize in conditions of steady state supply of sulphate (200 μM) and after sulphate depletion. Grain yield (100 kg ha^{-1}): A = 35; B = 60; A × B = 90; B × A = 100
Data are the means of 5 replications. Standard errors of means ranged from 5 to 12 percent of the values reported

Uptake conditions	Sterol content (μg g^{-1} root fr. wt)			
	A	B	A × B	B × A
Steady state	282	329	174	177
After depletion	288	256	132	124

Table 2. Sterol content, kinetic data of uptake, and internal pool of sulphate in the B × A maize hybrid. Data were obtained in conditions of regular nutrient supply after growth at different levels of sulphate. Fluxes are in nanomoles, internal pool in μmoles, sterol content in μg per g^{-1} root fresh weight.
Data are the means of 5 replications. Standard errors of means ranged from 5 to 12 percent of the values reported

	Sulphate concentration (μM) in the growth medium		
	50	200	400
Influx	159.7	197.3	299.7
Net uptake	101.9	93.6	39.9
Efflux	57.8	103.7	259.8
Sulphate pool	4.70	7.62	11.62
Sterol content	113.0	130.3	154.6

When the sterol content of plants grown at different sulphate concentration was plotted against the corresponding data of sulphate efflux (Fig. 1) a linear relationship with a high level of correlation was shown.

The data of the present paper, resulting from a preliminary approach, support the view of a role of sterols in the control of the efficiency of nutrient uptake by roots with special emphasis on the interconnection among low sterol content, more favourable ratio influx to efflux, and high grain yield.

Further insight into the involvement of sterols in the genetic and environmental regulation of

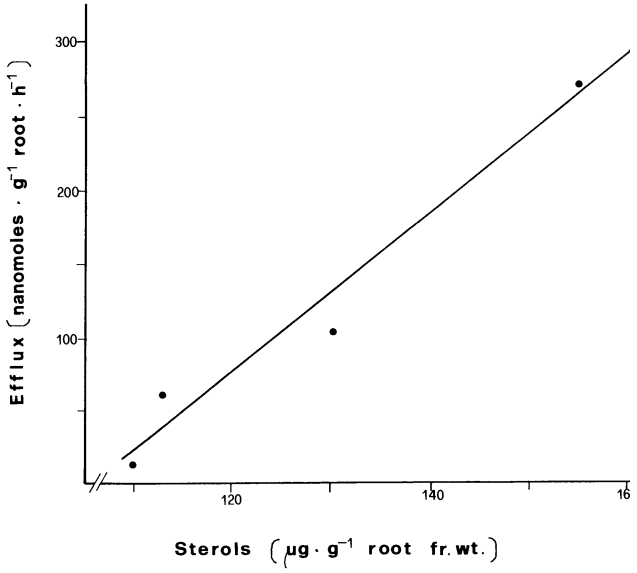

Fig. 1. Sterol content of the maize hybrid (B × A) at different values of sulphate efflux resulting from growth at different sulphate concentration.

nutrient uptake should be expected from studies on the distribution of sterols within the plasmalemma and tonoplast.

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PLSO PN 143
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Genetic differentiation in *Plantago major* L. in growth and P uptake under conditions of P limitation

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Key words: genetic differentiation, *Glomus fasciculatum*, growth analysis, P efficiency, *Plantago major*, vesicular-arbuscular mycorrhiza

Abstract

To study possible adaptive mechanisms inbred lines from three populations of *Plantago major* from sites that were found to differ in P availability were compared. In a pot experiment the growth and P uptake either in the presence or absence of *Glomus fasciculatum* was determined. Under these P-limited conditions it was shown by partitioning the relative growth rate (RGR, in $mg\,g^{-1}\,day^{-1}$) in the components root weight ratio (RWR, in $g_{roots}\,g_{plant}^{-1}$), specific P uptake rate (SPUR, in $\mu mol\,P\,g_{roots}^{-1}\,day^{-1}$), and P-efficiency (PEFF, in $mg\,\mu mol\,P^{-1}$), that the increase in RGR of mycorrhizal infected plants was related to an increase in SPUR, and a decrease in RWR and PEFF. *P. major* ssp. *major* had a lower RGR (related to a lower PEFF and SPUR) and a higher RWR than *P. major* ssp. *pleiosperma*. In a second experiment three inbred lines were compared upon P depletion in a nutrient solution. The *P. major* ssp. *major* line had a lower RGR and higher RWR, and a higher accumulation of P in the roots than the *P. major* ssp. *pleiosperma* lines under optimal growing conditions. There were no differences among the inbred lines in the relative contribution of inorganic P to the total P concentration in the shoot. The results are discussed in relation to the characteristics of the habitats of the investigated *P. major* populations.

Introduction

Plant growth in natural ecosystems is frequently limited by P availability (Chapin, 1980). Plants may evolve a number of plastic responses resulting in increased P uptake (see Clarkson, 1985 for a review). Mathematical models have shown that the uptake of phosphate ions—which are predominantly transported to the roots by diffusion—is greatly enhanced by an increase in the absorbing surface of the roots (Silberbush and Barber, 1983). The development of root hairs and/or the infection of vesicular-arbuscular mycorrhizal (VAM) fungi in roots can be considered as effective forms of root extension.

Adaptation of plant species to nutrient stress may occur in natural ecosystems. These adaptations may be based on a high uptake rate of the limiting nutrient, and/or a high nutrient efficiency, the inverse of tissue concentration. Surprisingly however, species from nutrient-poor habitats generally possess lower nutrient efficiencies compared to species from nutrient-rich habitats, when both are grown under identical conditions (Blair and Cordero, 1978; Chapin and Bieleski, 1982; Christie and Moorby, 1975). This suggests that nutrient efficiency is not an important adaptive mechanism under conditions of nutrient limitation. One of the explanations for this phenomenon may be that species from nutrient-poor habitats store nutrients in excess of immediate growth requirements (luxury consumption) during nutrient flushes. These nutrients may be mobilized in periods of nutrient stress. In this way, species from

nutrient-poor habitats may be able to produce more biomass in the annual cycle (Chapin, 1980).

The purpose of the present work was to find possible genetic differences within one species, which could be of adaptive value under conditions of P limitation. *Plantago major* was chosen as the study object since in this species genetic differences have been found to occur for several morphological and physiological characteristics (Van Dijk, 1984; Kuiper, 1983).

In a first pot experiment inbred lines of *P. major* from three populations (which differed in P availability) were compared in growth and P uptake either in the presence or absence of VAM fungi. In a second experiment growth and P fractions of three selected inbred lines were determined after a period of P stress.

Materials and methods

Field material

Plantago major L. ssp. *pleiosperma* Pilger populations from sites 'Angeren' (ANG) and 'Oostvoornse Meer' (OM), and *Plantago major* L. ssp. *major* population from site 'Westduinen' (WS) were studied. Site ANG is a riverbank which is flooded in winter. Site OM is a young dune valley, which is partly inundated in winter. Site WS is an old coastal grassland. Total P in the 0—15 cm soil layer at the sites are 10.5, 1.6 and 3.1 ppm respectively. Nine individual *P. major* plants were sampled per site in September 1986.

The dry weights of shoots were determined, and concentrations of total P, total N, Na, K, Ca, Mg were determined as described by Troelstra (1983), and ethanol-soluble sugars and starch concentrations as described by De Visser and Lambers (1983). Roots of the sampled plants were stained with Chlorazol black for assessment of mycorrhizal infection (Brundrett *et al.*, 1984). Percentages of mycorrhizal infection were determined by counting approximately 100 root pieces per sample using the gridline intersect method (Giovanetti and Mosse, 1980).

Pot experiment (Exp. 1)

A calcareous sandy soil (pH 8.5), taken from the 5—25 cm layer at site OM was used. After sieving, the soil was sterilized using γ-irradiation (2.5 MRad). One gram of $Ca_{10}(PO_4)_6(OH)_2$ was added per kg of dry soil, and mixed. Previous experiments had shown that this amount of P limited plant growth (Baas *et al.*, 1989).

Infected root fragments and spores from a pot culture of *Glomus fasciculatum* (Thaxt. sensu Gerdemann) Gerdemann and Trappe on white clover were collected, and 2.25 g inoculum (containing approximately 850 spores) was mixed per kg of soil (+VAM treatment). Sterilized root fragments were used in the control treatment (−VAM treatment). Plastic pots were used containing 600 g soil, and covered with tinfoil to reduce contamination and evaporation during the experiment.

Seeds of *P. major* inbred lines of populations ANG, OM, and WS were germinated on moistened glass beads. The three inbred lines per population had been obtained by self-fertilization as described by Van Dijk (1984). After germination two seedlings of each line were planted per pot through holes in the tinfoil. Initial seedling fresh weight of each inbred line was determined before planting. In the experimental design therefore inbred lines were nested within populations, and crossed with mycorrhizal treatments. There were five replicate pots per treatment. The pots received 10 mL of a nutrient solution with the following concentrations of macronutrients weekly (mM): NO_3^-, 60; SO_4^{2-}, 8; K^+, 20: Ca^{2+}, 20: Mg^{2+}, 8. Micronutrient concentrations were (μM): NaFeEDTA, 83; H_3BO_3, 369; $MnCl_2 \cdot 4H_2O$, 73; $ZnSO_4 \cdot 7H_2O$, 6; $CuSO_4 \cdot 5H_2O$, 0.3; $Na_2MoO_4 \cdot 2H_2O$, 0.5. The water content during the experiment was kept between 10 and 20% (w/w) using demineralized water. Pots were placed in a greenhouse under natural daylight conditions and a temperature of 23°C.

Plants were harvested after 49 days. Fresh and dry weights (after 48 h drying at 70°C) of shoots and roots were determined. Root subsamples were used to determine mycorrhizal infection as described above. Total P concentrations in shoot and roots were determined.

Water culture experiment (Exp. 2)

Seeds of three inbred lines (ANG3, OM3, and WS2; see Exp. 1) were germinated as in Exp. 1.

After germination 24 seedlings of each inbred line were placed on 10.4 l containers and pretreated with a 1/8 strength of the nutrient solution as used in Exp. 1, including KH_2PO_4 (0.25 mmol L^{-1}). The nutrient solution was replenished twice a week. The pH of the nutrient solution was adjusted to 6.0 every other day. Plants were grown in a greenhouse at 23°C under natural light conditions. After 16 days, plants were weighed (to determine RGR), and placed either on the same nutrient solution (12 plants; +P treatment), or on a nutrient solution without KH_2PO_4 (12 plants; −P treatment). The experiment therefore had a two-factorial design with inbred lines and P treatment as factors. After 30 days plants were harvested. Roots were washed in demineralized water, and fresh and dry weights of six plants of each treatment were determined. Total P was determined in these plants. The remaining six plants per treatment were freeze-dried, and the partitioning of inorganic, nucleic acid, ester and lipid P fractions was determined in the shoot according to Chapin and Bieleski (1982).

Calculations and statistical analyses

Relative growth rate (RGR) was calculated using the initial seedling weight of the inbred lines in Exp. 1, and the individual plant weights at the start of the +P/−P treatment in Exp. 2.

It is assumed that under stable nutrient-limited conditions biomass is proportionally related to nutrient uptake (Ingestad and Lund, 1986). Therefore, RGR (in mg FW g FW^{-1} day^{-1}) under P-limited conditions can be described as:

$$1/W\ dW/dt = 1/W\ d(P\ uptake)/dt \times W/(P\ uptake) \quad (1)$$

in which W = total plant weight, and P uptake = total accumulated P in the plant (calculated from shoot and root dry weights and respective P concentrations). The parameter W/(P uptake), the inverse of plant P concentration, is referred to as P-efficiency (PEFF, in mg FW μmol^{-1}), and 1/W d(P uptake)/dt as the P uptake rate, which may be divided into the root weight ratio (RWR, in g_{roots} FW g^{-1}_{plant} FW), R/W, and the specific P uptake rate (SPUR, in μmol P g^{-1}_{roots} FW day^{-1}), 1/R d(P uptake)/dt according to:

$$1/W\ d(P\ uptake)/dt = 1/R\ d(P\ uptake)/dt \times R/W \quad (2)$$

Combining Eqs. (1) and (2) gives:

$$1/W\ dW/dt = 1/R\ d(P\ uptake)/dt \times R/W \times W/(P\ uptake) \quad (3)$$

$$\text{or } RGR = SPUR \times RWR \times PEFF$$

The SPUR was calculated in Exp. 1 using Eq. (3).

Analysis of variance (ANOVA) was performed using populations, mycorrhizal treatment, and inbred lines (nested within populations) as independent variables in Experiment 1, and inbred lines and P-treatments in Experiment 2. General contrasts between *P. major* ssp. *pleiosperma* (ANG and OM) and *P. major* ssp. *major* (WS), and within ssp. *pleiosperma* were calculated by partitioning the sum of squares of populations (Exp. 1) or of the inbred lines (Exp. 2). If variances were not homogeneous according to Cochran's Q test, data were either ln(x) or $\sqrt{(x)}$ transformed prior to ANOVA. Tukey's test was used to test differences in field data, and in means of Exp. 2 at the significance level of 0.05.

Results

Field data

P. major plants from populations ANG, OM and WS differed in above-ground biomass production, mineral nutrient and starch and soluble sugar concentrations, and mycorrhizal infection (Table 1). The highest correlation between shoot biomass and mineral nutrient concentration was found for the total P concentration ($r = 0.59$). The lowest P concentration in the shoot and the highest percentage of mycorrhizal infection was found in OM.

Pot experiment (Exp. 1)

Mycorrhizal infection was established in the +VAM treatment at the end of the experiment (Table 2), and was lowest in population OM. Mycorrhizal infection increased dry weight in both shoot and roots. *P. major* ssp. *pleiosperma* however had higher shoot weights than *P. major* ssp. *major* in both treatments.

Table 1. Shoot dry weight (g), mineral nutrient (μmol g dry wt^{-1}), starch and sugar (mg g dry wt^{-1}) concentrations in the shoot, and mycorrhizal infection (%) of *Plantago major* ssp. *pleiosperma* (at sites ANG and OM) and ssp. *major* (site WS). Means (n = 9) followed by the same letter are not significantly different ($P < 0.05$)

	Site		
	ANG	OM	WS
Shoot dry weight	0.27 b	0.13 a	0.38 b
P	125 c	30 a	77 b
N	2903 c	814 a	1265 b
K	719 b	804 b	352 a
Na	327 c	57 b	19 a
Ca	746 a	1219 c	1088 b
Mg	103 a	271 c	197 b
Starch	72 a	129 b	92 a
Sugars	23 a	129 c	92 b
% VAM	8 a	46 b	12 a

The dry matter content in shoot and roots was slightly lower in the +VAM treatment ($P < 0.05$). There were significant differences between inbred lines (within populations) in dry matter content in shoot and roots.

In both shoot and roots P concentrations were increased in the +VAM treatment. Besides, differences in shoot P concentration both between the subspecies and within the ssp. *pleiosperma* population were apparent.

Since both dry weights and P concentrations were increased, the P uptake also was increased in the +VAM treatment. A smaller fraction of this P uptake was present in the roots in the +VAM treatment. The *P. major* ssp. *major* population WS had a larger fraction of the P uptake in the roots than both *P. major* ssp. *pleiosperma* populations.

From Figure 1 — in which characteristic population differences from Table 2 are shown — the inbred line differences (within populations) can be seen to be relatively small.

The relative growth rate (RGR) was increased in the +VAM treatment (Table 3). The root weight ratio (RWR) and the P-efficiency (PEFF), both components of the RGR, were lower in the +VAM treatment. However, the increased RGR in the +VAM treatment was related to a more than doubled specific P uptake rate (SPUR).

Despite the higher RWR *P. major* ssp. *major* did not have a higher RGR than *P. major* ssp. *pleiosperma* both in the +VAM and the −VAM treatments. This was related to both a lower SPUR and a lower PEFF. The difference in RGR between *P. major* ssp. *pleiosperma* populations ANG and OM could only be related to a difference in SPUR.

Water culture experiment

As a result of P depletion, growth and P concentrations in both shoots and roots were decreased, and the RWR and PEFF were increased

Table 2. Means (n = 15) and ANOVA table of mycorrhizal infection (%) dry weight (g pot^{-1}), percentage dry matter (%), P concentration (μmol P g dry wt^{-1}), P uptake (μmol pot^{-1}) and fraction of P uptake in roots (%) of inbred lines of *Plantago major* populations in mycorrhizal (+VAM) and non-mycorrhizal (−VAM) treatment after 49 days in experiment 1. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Population Treatment		VAM %	Dry weight		Dry matter (%)		P		P uptake	P uptake roots (%)
			Shoot	Roots	Shoot	Roots	Shoot	Roots		
ANG+VAM		70	1.28	0.46	16.9	8.3	34	44	61	31
OM		50	1.34	0.48	14.7	7.9	38	42	68	29
WS		63	1.03	0.49	16.8	8.4	41	45	61	35
ANG−VAM	—	0.20	0.12	18.5	10.3	28	30	9	41	
OM		—	0.28	0.18	14.5	8.5	29	29	13	40
WS		—	0.12	0.11	18.5	10.5	30	31	7	47
ANOVA table	df									
Population (Pop)	2/6									
between subspecies	1/6	ns	**	ns	ns	ns	*	ns	*	*
within ssp. *pleiosp.*	1/6	**	ns	ns	*	ns	*	ns	*	ns
VAM	1/6	—	***	***	*	*	***	***	***	***
Pop × VAM	2/6	—	ns	*	ns	ns	ns	ns	ns	ns
inbred line (L)	6/67	ns	ns	*	***	***	ns	ns	ns	ns
L × VAM	6/67	—	ns	ns	ns	**	ns	ns	ns	ns

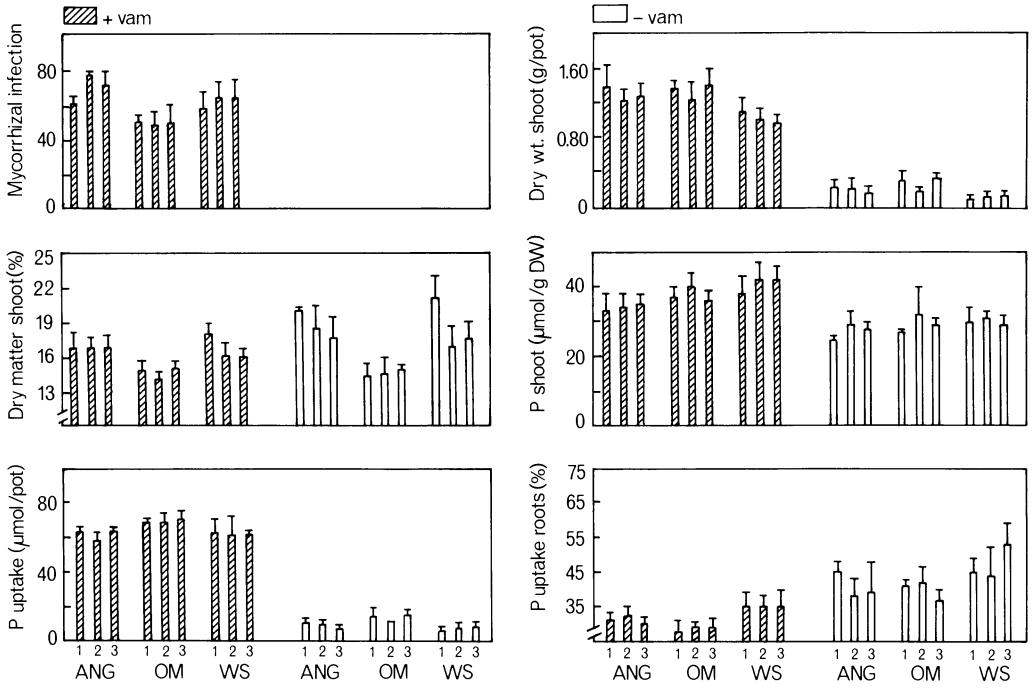

Fig. 1. Mycorrhizal infection, dry weight, percentage dry matter and P concentration in shoot, total P-uptake and relative contribution of P-uptake in roots of *Plantago major* ssp. *pleiosperma* (ANG, OM) and ssp. *major* (WS) inbred lines in mycorrhizal (+VAM) and non-mycorrhizal (−VAM) treatments of Experiment 1. *Bars* indicate s.d. (n = 5).

Table 3. Means (n = 15) and ANOVA table of relative growth rate (RGR, in mg fr. wt g fr wt^{-1} day^{-1}), root weight ratio (RWR, in g_{roots} fr. wt g_{plant} fr. wt^{-1}), P-efficiency (PEFF, in mg fr. wt. μmol P^{-1}) and specific P uptake rate (SPUR, in μmol P g_{roots} fr. wt^{-1} day^{-1}) of *Plantago major* populations in mycorrhizal (+VAM) and non-mycorrhizal (−VAM) treatment in experiment 1. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Population	Treatment	$RGR_{0-49\ days}$	RWR	PEFF	SPUR
ANG	+VAM	164	0.40	203	2.0
OM		148	0.38	209	1.9
WS		146	0.47	184	1.7
ANG	−VAM	127	0.51	241	1.0
OM		120	0.50	288	0.9
WS		103	0.59	232	0.8
ANOVA table	df				
Population (Pop)	2/6				
between subspecies	1/6	**	***	*	**
within ssp. *pleiosp.*	1/6	*	ns	ns	*
VAM	1/6	***	***	***	***
Pop × VAM	2/6	*	ns	ns	ns
inbred line (L)	6/67	***	**	***	ns
L × VAM	6/67	ns	ns	*	ns

(Table 4; $P < 0.001$). The fraction of P uptake in the roots to the total P content had increased in the −P treatment.

All the plant properties investigated differed in the inbred lines. *P. major* ssp. *major* line WS2 had a lower RGR and higher RWR in the +P treatment than the *P. major* ssp. *pleiosperma* lines. Besides, this line had a higher fraction of P uptake in the roots, and a lower PEFF than the *P. major* ssp. *pleiosperma* lines.

Table 4. Means (n = 6) and ANOVA table of dry weight (g), P concentration (μmol P g dry wt^{-1}), fraction of P uptake in roots (%), relative growth rate (RGR, in mg fr. wt g fr. wt^{-1} day^{-1}), root weight ratio (RWR, in g_{roots} fr. wt g_{plant} fr. wt^{-1}) and P-efficiency (PEFF, in mg fr. wt μmol P^{-1}) of *P. major* inbred lines (ssp. *pleiosperma* ANG3, OM3 and ssp. *major* WS2) of the +P and −P treatments in experiment 2. Means followed by the same letter are not significantly different. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Line	Treatment	Dry weight		P		P uptake roots (%)	$RGR_{16-30\ days}$	RWR	PEFF
		Shoot	Roots	Shoot	Roots				
ANG3	+P	1.26 bc	0.19 abcd	224 c	237 cd	11 a	208 b	0.22 a	43 a
OM3		1.75 c	0.24 bcd	224 c	209 c	12 a	204 b	0.20 a	55 a
WS2		0.75 a	0.13 ab	202 c	340 d	27 b	171 a	0.34 b	39 a
ANG3	−P	0.47 a	0.17 abc	32 a	52 ab	38 b	—	0.42 c	223 c
OM3		0.74 ab	0.28 cd	26 a	36 a	35 b	—	0.45 c	326 d
WS2		0.20 a	0.10 a	44 b	58 b	32 b	—	0.42 c	133 b
ANOVA table	df								
inbred line (L)	2/30								
between subspecies	1/30	***	***	ns	***	*	***	***	***
within ssp. *pleiosp.*	1/30	**	**	*	*	ns	ns	ns	***
P	1/30	***	ns	***	***	***	—	***	***
L × P	2/30	ns	ns	***	ns	***	—	***	***

The relative contribution of the different P fractions to the total P concentration in the shoot of both the +P and the −P treatments is given in Figure 2. Inorganic P (Pi) contributed 56—61% in the +P treatment, and 44—49% in the −P treatment. Nucleic acid P contributed 20—22% in the +P treatment and 25—31% in the −P treatment. The lipid P and ester P fractions were in the same range with 8—11% and 9—16% for the +P and −P treatments, respectively. The reduction of the relative contribution of Pi to total P as a result of the −P treatment was highly significant ($P < 0.001$). However, line or line × P treatment interaction effects were not significant for the Pi fraction.

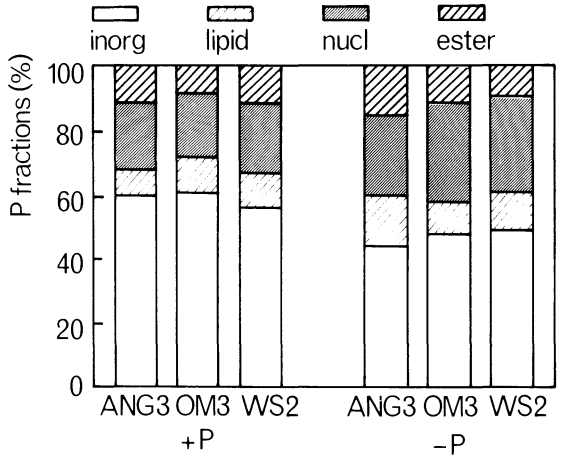

Fig. 2. Relative contribution of inorganic (inorg), lipid, nucleic acid (nucl) and ester fractions to total P in *P. major* ssp. *pleiosperma* inbred lines (ANG3, OM3) and *P. major* ssp. *major* inbred line WS2 in the +P treatment and −P treatment from Experiment 2.

Discussion

Effects of mycorrhizal infection

Mycorrhizal infection clearly had increased dry weight and the P concentrations in both shoot and roots, and the P-uptake of the inbred lines of *Plantago major* populations ANG, OM, and WS (Table 2). Similar results with other plant species are generally found under P-limiting conditions (*e.g.* Gianinazzi-Pearson and Gianinazzi, 1983).

To get more insight into the cause of the differences in growth in the experiment where P was limiting growth, the relative growth rate (RGR) was divided into the components root weight ratio (RWR), P-efficiency (PEFF), and specific P uptake rate (SPUR). The RWR and PEFF as measured at the end of the experiment may possibly not have been constant in time. Although the latter may result in an over- or underestimated SPUR as determined by Equation (3), differences between inbred lines are supposed to be affected in a similar manner.

It could be demonstrated that the increased RGR in the +VAM treatment was only due to an

increased SPUR, since both the P-efficiency and the RWR were decreased (Table 3). An increased SPUR due to mycorrhizal infection also was found in soybeans (Pacovsky and Fuller, 1986), onion (Sanders and Tinker, 1973) and may be ascribed to a more effective exploration of the soil for available P by the extraradical hyphae of the VAM fungus.

Population differentiation

In the present work possible adaptations to P-limitation in *P. major* were studied. Therefore, *P. major* populations were selected in which the dry matter production was clearly different. Chemical analysis of shoot material showed low P and high carbohydrate concentrations at site OM (Table 1), which indicates that P may be limiting. Fertilization with P indeed increased growth of *P. major* ssp. *pleiosperma* at this site (Baas, unpublished results). It was therefore hypothesized that OM inbred lines would be more adapted to P-limitation than ANG or WS inbred lines.

In the pot experiment OM showed less mycorrhizal infection than ANG or WS (Table 2). The high mycorrhizal infection percentage at site OM (Table 1) therefore is probably the result of the low P concentration in the shoot of plants from this population, since mycorrhizal infection is determined among others by the internal P concentration (Sanders, 1975).

The mycorrhizal dependency, defined as the dry weight of + VAM plants divided by the dry weight of − VAM plants (Gerdemann, 1975) was for ANG, OM, and WS 5.4, 4.0 and 6.6 respectively. The smaller growth response of OM to the + VAM treatment may have been due to the lower degree of infection.

Although OM produced more shoot biomass and had a higher P uptake, the RGR of this population was lower than the RGR or ANG (Table 3). Therefore, *P. major* from site OM presumably had not adapted to conditions of P-limitation by means of a high RGR.

Within *P. major* the subspecies *pleiosperma* and *major* can be distinguished (Mølgaard, 1976). *P. major* ssp. *pleiosperma* generally is annual, and — in the terminology of Grime (1977) — has a ruderal strategy compared to the more perennial ssp. *major*.

In the pot experiment, *P. major* ssp. *major* population WS had a higher RWR, but not a higher RGR than *P. major* ssp. *pleiosperma* populations ANG and OM. This therefore indicates that a higher RWR is not necessarily a beneficial factor in the growth under P-limiting conditions, as has been suggested (Chapin, 1980). A higher RGR of *P. major* ssp. *pleiosperma* — also found in the water culture experiment at high nutrient availability — has been reported before (Dijkstra and Lambers, 1986; Kuiper, 1983; Poorter and Lambers, 1986), and was achieved due to both an increased PEFF and an increased SPUR.

Gerloff and Gabelman (1983) suggest several mechanisms for genotypic variation in plant growth during nutrient limitation. Mechanisms which could possibly explain the differences in P-efficiency are differences in the ability to release stored inorganic P (Pi) from the vacuole, and differences in the capacity for maintaining an active metabolism at low tissue concentrations. It is estimated that 85 — 95% of total Pi in the plant is present in the vacuole, where it is metabolically inactive (Bieleski, 1973). Therefore, differences in percentages of Pi in the vacuole might explain differences in P-efficiency between the subspecies. In Experiment 2 the contribution of the different P fractions in the shoot, including Pi, was investigated in inbred lines A3, O3 and W2, under non-limiting conditions, and after a period of P depletion. The results (Fig. 2) show that upon P-depletion the relative contribution of Pi decreased. The contribution of the different fractions to total P is within the same range as was found for barley (Chapin and Bieleski, 1982). No significant differences between the inbred lines were found in relative contribution of Pi to the total tissue P concentration. This is in accordance with results obtained with Chionochloa species from habitats differing in P availability (Chapin *et al.*, 1982). It therefore indicates that differences in P-efficiency are not the result of differences in storage of Pi.

One of the possible causes of the occurrence of *P. major* ssp. *pleiosperma* at the nutrient-poor site OM is the relatively short growing season, due to inundation in the winter. A high rather than low RGR under nutrient-limited conditions, maintained by a high nutrient use efficiency, followed by reproduction may be an advantage under these conditions.

A low nutrient use efficiency, and consequently, a low RGR by the more perennial *P. major* ssp. *major* may be an advantage in so far that nutrients are retained, especially in the roots, to be used for growth in the subsequent spring.

Acknowledgements

We thank L A P Lotz for providing inbred lines of *P. major*, J M M van Damme, A H Freijsen, S R Troelstra and J W Woldendorp for comments on the manuscript, H Koelewijn for statistical advice, W Smant for chemical analyses and N W Tolmeijer for drawing the figures.

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Session 2

Genotypic responses to a) water stress, b) salinity and c) acidity and deficiency or excess of elements

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 137–143.
PLSO PN 144
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Physiological characteristics responsible for drought resistance in different pea cultivars

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Key words: drought resistance, grain yield, harvest index, leafless, leaf relative water content, leaf water potential, osmotic adjustment, pea, productivity, semi-leafless, water stress, water use efficiency

Abstract

We have studied some aspects of their drought resistance in different pea cultivars and segregating families. Two genotypes were semi-leafless (leaflets transformed in tendrils, big stipules), and one was leafless (leaflets transformed in tendrils, small stipules). This attempt has been made in the laboratory, where we looked for osmotic adjustment, and in the field, where we studied the grain yield when the plants were subjected to different degrees of water stress. Withholding water to the plants, we found, in the laboratory, good correlations between leaf relative water contents and leaf water potentials. This allowed us to calculate the relative water content values for one fixed value of water potential: -1.1 MPa (this was near the irreversible wilting point for the plants in our conditions). The higher the relative water content of the genotype, the better must be its osmotic adjustment.

The two genotypes exhibiting the best productivity, when stressed in the field (one of them was semi-leafless), and also another semi-leafless genotype, with reasonably good yield, had a poor performance as osmotic adjusters. On the other hand, two conventional leaf genotypes also showed a good yield in stress conditions, and osmoregulation capacity. From this it seems that peas have, among possibly, many others, two mechanisms to face drought: one morphological, reducing their transpiration surface, and so showing a better water economy; and one physiological, that induces a certain degree of osmotic adjustment.

It is also interesting that a good positive linear correlation was found between harvest index, and grain yield under stress (leafless and semi-leafless genotypes were excluded). From a practical point of view we can say that the inclusion of the semi-leafless character (responsible: one recessive gene), into high harvest index genotypes (under stress), will certainly produce optimum drought resistant cultivars.

Introduction

Through this work we have tried to study in different pea cultivars and segregating families, some aspects of their drought resistance. Dry grain yield was evaluated on ten pea genotypes (*Pisum sativum* L.), grown in the field under different irrigation regimes. The same genotypes were grown in pots, in climatic chambers, and at a certain point of their growth cycle, were drought stressed by withholding water. The pot grown plants while being stressed, were assayed for water potential and relative water content to make an estimate of their capability for osmotic adjustment. The experiments that are described below cover some aspects of one research project in which we try to find correlations between field yield under water stress conditions and biochemical, physiological, anatomical or morphological parameters that may indicate drought resistance, or rather acceptable yields in limiting water conditions. Our last target is to bring our results to breeders, so that they can eventually

be used to obtain new resistant cultivars. Trying to obtain a better water use efficiency, the use of biochemical, physiological and anatomical characteristics, usually implies characterising techniques too tedious to be used in a routine way (Zobel, 1983). The objective, may then be, to obtain good correlations between anatomical, physiological, and biochemical characteristics, and others of an agronomic or morphological nature, usually used in breeding programs, and then to improve for the correlating characters, instead of doing it for those directly required. To this type of process Zobel (1983), has called associative breeding. Recently, some authors (Blum, 1983; 1985; Ceccarelli, 1984; Morgan, 1984; Sullivan and Jordan, 1987), have revised some of the characters, that in principle must be responsible for inducing drought resistance in plants, and the techniques to examine the variability of those characters.

Materials and methods

The following genotypes were used:

Reference	Name, origin and characteristics
V1	F5-mass (Frisson × Filby), leafless.
V2	F5-mass (Frisson × Filby), semi-leafless.
V3	Local ecotype from Spain, conventional leaves.
V4	Frisson, commercial cultivar, conventional leaves.
V6	Desso, commercial cultivar, conventional leaves.
V8	Amino, commercial cultivar, conventional leaves.
V9	Fride, commercial cultivar, conventional leaves.
V10	Local ecotype from Spain, conventional leaves.
V12	F5-mass (Desso × Filby), semi-leafless.
V13	F5-mass (Lysima × Frisson), conventional leaves.

The semi-leafless character indicates that leaves have their leaflets transformed in tendrils, this character is due to the recessive gene af. The leafless character besides having the leaflets transformed in tendrils, presents also a very reduced size of the stipules. This character is due to the action of the recessive gene st. According to that, semi-leafless and leafless genotypes are respectively: afaf StSt and afaf stst (Snoad, 1981).

The field experiment was carried out in the experimental farm 'La Canaleja' (Madrid), on a calcic-haploxeralf soil. Sowing date took place in January 22–23, 1987. Maximum and minimum temperatures, and rainfall registered during the pea growth period are indicated in Figure 1. The pea genotypes were sown in lines perpendicular to line source sprinklers, so that plants received decreasing amounts of water, the farther they were from the line sprinkler (Hanks *et al.*, 1976). Peas from each genotype were sown in six lines 0.30 m apart, and the population density was of 696,000 plants ha^{-1}. Four replications were made for each variety, two were situated to the north of the line source sprinkler, and two were to the south. Yield results were analysed, by means of a strip split plot design of analysis of variance (Cochran and Cox, 1957). Irrigation was provided to replace the weekly evapotranspirated water, to the plants next to the sprinkler line. The estimate of evapotranspiration was the mean of the measurements made on genotypes V1 and V3 (which showed a poor and a high vegetative growth respectively), with the help of a neutron probe Nardeux, mod. solo-20. Table 1 gives the irrigation doses received by the pea genotypes in relation to the distance of the plants to the line sprinker. Four sets of six catch cans were used to control the amounts of irrigation received every two meters. Fertilization was: N, 30 kg ha^{-1}; P_2O_5, 80 kg ha^{-1}; K_2O, 140 kg ha^{-1}. Plots were hand weeded. Plant evapotranspiration at 12 and 14 m of the sprinkler line has been calculated as the

Table 1. Amount of water received by the pea genotypes in relation to the distance of the plants to the line sprinkler

Distance to the sprinkler line (m)	Irrigation water (mm)
4	214
6	195
8	121
10	44
12	12
14	3

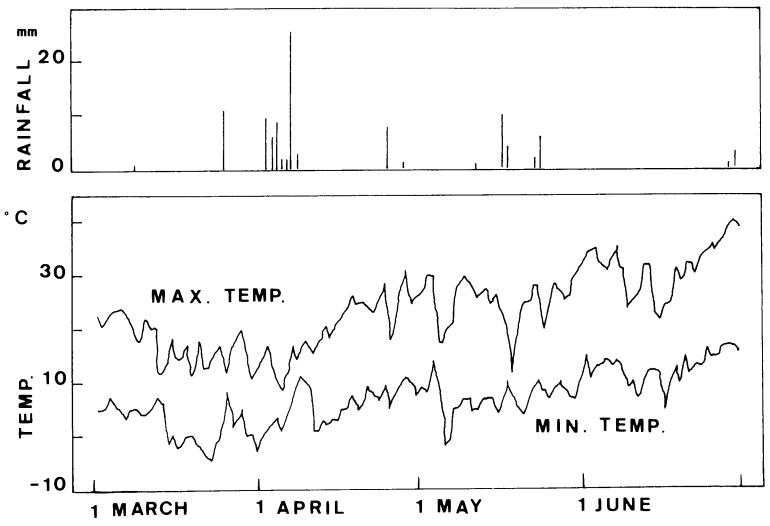

Fig. 1. Maximum and minimum temperatures, and rainfall registered during the pea growth period.

addition of: 100 mm H_2O that correspond in both cases to the estimate of the root-soil water reserve (Elías, 1977), plus total registered rainfall during March, April, May and mid June, plus the estimate of irrigation water received at each distance. Laboratory experiments were made on plants grown in climatic chambers, in pots of 10.5 cm diameter, where vermiculite was used as substrate. After germination plants were irrigated with a complete nutritive solution, being one out of four irrigations only with water. Photosynthetic active radiation was 317 $\mu E\,m^{-2}s^{-1}$, measured at the top of the plants, using a radiation sensor Li-cor, mod LI-190SB and radiometer Li-cor, mod. LI-185B. Photoperiod was of 16 hours light and 8 hours dark, and temperature was 21 and 16°C, during the day and night respectively. Water was withheld to the pea plants when they were 25-days old. Leaf water potential (ψ), was measured in the successive days, by means of a pressure bomb, Soilmoisture, mod. 3005. Leaf relative water content (RWC), was also estimated using floating leaf discs, over distilled water, for 16 hours approximately, at 1°C, in the dark, for rehydration (Milburn and Weatherley, 1971). To measure leaf water potential and relative water content, the second leaf of each plant was used, considering as first the one completely developed nearest to the apex. Water potential was measured on leaflets, and RWC upon three or four discs from the complementary leaflet of each leaf. When measures were taken on semi-leafless genotypes, the estipules were used instead of leaflets. On conventional leaf genotypes, it was verified that, water potential and relative water content values did not differ significantly, when made either on stipules or leaflets from the same leaf. Three replications were made for each measure of leaf water potential and relative water content.

Results

In the Table 2, grain yields ($g/1.2\,m^2$), are represented, as a function of the distance to the line sprinker (each value is the mean of four). The volume of water received (dm^3), per harvested elementary plot ($1.2\,m^2$) is also indicated. Good linear correlations ($Y = a + bX$) can be found between grain yield (Y), and the volume of irrigation water for the different treatments (distances to the line sprinkler). Regression straight lines are shown in Table 3. Using the lines from Table 3, we have built two rankings in which the different genotypes are disposed in an orderly way, according to their potential yield (for an amount of irrigation water, mean of the ones received at 4 and 6 m from the line sprinkler), and stress yield (for an amount of irrigation water, mean of the ones received at 12 and 14 m from the line sprinkler). These lists are indicated in Table 4. Table 5 shows the harvest indexes (HI, dry grain weight per total

Table 2. Grain yield (g/1.2 m^2), of the different genotypes, as a function of the distance to the line sprinkler. The registered irrigation water per elementary harvested plot (1.2 m^2) is also shown. LSD (α = 0.05) = 73.99

Distance to sprinkler line (m)	Yield										Irrigation water (dm^3/1.2 m^2)
	Genotypes										
	V1	V2	V3	V4	V6	V8	V9	V10	V12	V13	
4	386	509	199	486	673	467	427	166	569	555	257
6	335	486	219	402	618	493	469	193	554	575	234
8	250	403	229	391	570	430	390	097	464	477	145
10	181	315	172	268	383	315	269	198	343	320	53
12	178	235	194	226	300	271	260	221	291	231	14
14	203	247	178	220	260	238	281	162	272	262	3

aboveground dry matter weight), of the different genotypes at 4, 6, 12 and 14 m from the line sprinkler. Using some of the data shown in Table 5, one ranking can be established for the different genotypes, that are so classified according with the value of their harvest indexes in drought stress conditions (mean of the harvest index at 12 and 14 m from the line sprinkler). A good linear correlation ($Y = a + bX$, $R^2 = 0.89$, $a = 30.57$, $b = 449.54$), was obtained between stress grain yield (Y, g) for the different genotypes (Table 4) and the corresponding values of harvest index (in drought stress conditions), whenever leafless and semi-leafless genotypes (V1, V2 and V12), are excluded. In Table 6, water use efficiency (WUE, aboveground dry matter per evapotranspirated water, g dm^{-3}) is indicated for the pea genotypes assayed and water stress conditions, 12 and 14 m from the line sprinkler. With data from Table 6, one ranking or orderly list of the different genotypes according to their WUE can be constructed.

In the experiment carried out in growth chambers, parabolic correlations ($Y = a + bX + cX^2$), were found between relative water content (Y) and leaf water potential (X, MPa, Table 7). Similar correlations were found by Morgan, for wheat in 1983. If in the regression lines, shown in Table 7 we calculate the RWC for a prefixed leaf water potential of -1.1 MPa, that is near the permanent wilting point of the pea plants in our experimental conditions, we can make an evaluation, of the water status, and indirectly of the osmotic adjustment of each genotype, (the genotypes showing a higher capacity to adjust osmotically, will appear with a better water status). In Table 8, a ranking is shown in which the different genotypes are arranged in a list of decreasing RWC values.

Table 3. Regression lines ($Y = a + bX$), of grain yield (Y, g), over the volume of irrigation water (X, dm^3/1.2 m^2), received by the different treatments (distances to the sprinklers)

Genotype	R^2	a	b	Potential yield (g)	Stress yield (g)
V1	0.92	167.36	0.75	351	174
V2	0.99	240.93	1.06	501	250
V3	0.40	183.59	0.13	215	185
V4	0.95	219.18	0.96	455	228
V6	0.96	284.90	1.55	665	299
V8	0.95	257.48	0.95	490	266
V9	0.91	257.43	0.78	449	264
V10	0.06	184.33	−0.10	160	184
V12	0.99	276.36	1.18	566	287
V13	0.96	245.70	1.34	574	258

Discussion and conclusions

The existence of positive linear correlations, almost in every genotype, between grain yield and irrigation doses indicates that in 1987, unirrigated

Table 4. Rankings of the assayed genotypes, according to the estimates of their potential (4–6 m distance from the line sprinkler) and stress yield (12–14 m from the line sprinkler)

Ranking	Genotype	Potential yield (g)	Ranking	Genotype	Stress yield (g)
1	V6	665	1	V6	299
2	V13	574	2	V12	287
3	V12	566	3	V8	266
4	V2	501	4	V9	264
5	V8	490	5	V13	258
6	V4	455	6	V2	250
7	V9	449	7	V4	228
8	V1	351	8	V3	185
9	V3	215	9	V10	184
10	V10	160	10	V1	174

Table 5. Harvest index for the different pea genotypes at the nearest (4–6 m) and the farthest distances (12–14 m) of the line sprinkler

Distance to sprinkler (m)	Harvest index									
	Genotypes									
	V1	V2	V3	V4	V6	V8	V9	V10	V12	V13
4	0.58	0.51	0.22	0.62	0.65	0.52	0.61	0.17	0.61	0.56
6	0.62	0.50	0.23	0.64	0.67	0.57	0.64	0.25	0.63	0.59
$\bar{X}$	0.60	0.51	0.23	0.63	0.66	0.55	0.63	0.21	0.62	0.58
12	0.53	0.43	0.35	0.53	0.58	0.49	0.55	0.36	0.54	0.49
14	0.54	0.42	0.35	0.43	0.55	0.47	0.56	0.32	0.51	0.48
$\bar{X}$	0.54	0.43	0.35	0.48	0.57	0.48	0.56	0.34	0.53	0.49

Table 6. Water use efficiency for the different genotypes and water stress conditions: 12 and 14 m from the line sprinkler

Distance to sprinkler (m)	WUE									
	Genotypes									
	V1	V2	V3	V4	V6	V8	V9	V10	V12	V13
12	1.35	2.16	2.20	1.71	2.08	2.18	1.87	2.46	2.16	1.86
14	1.58	2.44	2.10	2.11	1.98	2.13	2.08	2.09	2.22	2.27
$\bar{X}$	1.47	2.30	2.15	1.91	2.03	2.16	1.98	2.28	2.19	2.07

or poorly irrigated treatments bore the consequences of a certain drought stress, showing lower yields than the properly irrigated ones (Tables 2 and 3). From Table 4, it can be seen that the V6 genotype (commercial cultivar Desso) showed the best ranking position, both under water stress and in non stressing conditions. The semi-leafless genotype V12 (Desso × Filby) occupied also a good position in both circumstances. In the last positions, in both cases, with or without stress, we can find the genotypes V1, V3 and V10. Other genotypes situated at intermediate levels like V8 and V9 improve their positions when stressed, while V13, V2 and V4 descent in the ranking, under drought. Genotypes V3 and V10 are local ecotypes collected in Spain, never subjected to any genetic improvement and their poor results are therefore not surprising. Genotype V1 is leafless and probably due to its lack of assimilating surface presents so a poor performance. Perhaps its position could be improved at higher plant densities (Hedley and Ambrose, 1984). The excellent correlation between harvest index in water stress conditions, Table 5, and grain yield also under stress, Table 4, indicate that this agronomic character may be used to select

Table 7. Correlations ($Y = a + bX + cX^2$), between relative water content (Y, per cent), and leaf water potential (X, MPa), in different pea genotypes

Genotype	R^2	a	b	c
V2	1.00	86.39	37.67	−60.48
V3	0.94	93.13	1.81	−21.79
V4	0.98	90.30	5.37	−24.38
V6	0.87	84.40	22.27	−39.98
V8	0.93	74.40	49.27	−45.10
V9	0.99	94.93	−16.71	−8.33
V10	0.66	90.80	3.91	−23.53
V12	0.97	106.50	−74.12	23.25
V13	0.97	94.95	−19.01	−4.52

Table 8. Pea genotypes classified according to their decreasing relative water content values (RWC), for a pre-established leaf water potential (ψ), of −1.1 MPa

Ranking	Genotype	RWC (for $\psi = -1.1$ MPa)
1	V8	74.03
2	V3	68.75
3	V13	68.56
4	V4	66.71
5	V10	66.62
6	V9	66.48
7	V6	60.53
8	V2	54.65
9	V12	53.11

drought resistant pea genotypes. Other authors (Ceccarelli, 1984), have shown previously the importance of this trait in different species. Nevertheless it is important to notice that to obtain a high correlation coefficient (R^2), in that regression line, it was necessary to discard the leafless and semi-leafless genotypes, that sometimes may show contradictory characters in relation with the genotypes with standard leaves. For instance genotype V1 (leafless), has a high harvest index under stress (Table 5), nevertheless it shows a low yield (Table 4), and also a poor WUE (Table 6). Genotype V2 (semi-leafless), shows a low harvest index (Table 5), but its excellent WUE compensates, producing an acceptable yield under drought.

It is of interest to recall the model proposed by Passioura (1977): Yield = Evapotranspiration (ET) × Water use efficiency (WUE) × Harvest index (HI). This equation explains clearly why genotypes like V1 or V2 inevitably must lower the correlation coefficient when regressing yield over harvest index. In Table 6 we observe that two of the three most efficient water using genotypes are semi-leafless (V2 and V12); this seems reasonable as a reduced evapotranspiration surface must support a better water economy. Other authors (Wilson *et al.*, 1981) have obtained similar results. Returning to Passioura's model and assuming that their components are largely independent (Passioura, 1986), yield could be greatly improved for a fixed evapotranspiration value, by increasing harvest index and water use efficiency independently. We have shown that one means of improving the latter trait is by means of the semi-leafless character (Table 6), and as this is produced by one recessive gene it could be easily introduced into genotypes with a poor water use efficiency, but that otherwise show an elevated harvest index under stress. In relation to the alleged independence of harvest index and water use efficiency, Harvey (1977), has shown, working with semi-leafless and conventional peas, that leaf morphology did not affect the translocation potential or pattern of partitioning of assimilates in the plant. We conclude that the semi-leafless character was unlikely to be responsible for any great imbalance in dry matter accumulation on reproductive or vegetative organs. From Table 8 we can learn that the genotypes V6 and V12, that show an optimum yield under stress are practically the worst hydrated at $\psi = -1.1$ MPa, with slight symptoms of performing an efficient osmotic adjustment. On the other hand it is not possible to find a positive linear correlation between RWC (at $\psi = -1.1$ MPa), and yield under stress. Nevertheless two of the genotypes, V8 and V13 with greater possibilities of possessing the mechanism of osmotic adjustment (Table 8), show acceptable or even good yields both under irrigated and stressed conditions (Table 4). It is therefore possible that the mechanism of osmotic adjustment, although it does not seem to be a generalized one, for all the genotypes performing well under water stress, may play an important part in some genotypes (V8) with outstanding yield under drought (Table 4). In fact the presence of osmotic adjustment has been described in pea roots by Greacen and Oh (1972) and in general can be considered as a widespread mechanism of drought resistance (Morgan, 1984). Summing up we can say that a high harvest index under stress, together with an optimum water use efficiency, that in part can be achieved through the easily inherited semi-leafless character, are the keys to obtain optimum yields under stress. As for physiological parameters, the presence of osmotic adjustment, although possibly playing an important role in some cases, it will certainly be necessary to carry out more research.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 145–150.
PLSO PN 145
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Effects of water deficit on osmotic adjustment, photosynthesis and dry matter production of rice (*Oryza sativa* L.) genotypes

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Key words: osmotic adjustment, photosynthesis, rice, water deficit

Abstract

Three genotypes of upland and one genotype of lowland rice (*Oryza sativa* L.) were transplanted in pots, and were exposed to gradually developed long-term, and rapidly developed short-term water deficits. Genotypical difference in osmotic adjustment varied with the development of stress. Under long-term water deficits, the three upland rice genotypes showed the higher degree of osmotic adjustment, and thus maintained higher turgor, than that of lowland genotype. With short-term water deficit, upland rice genotypes 4652 and Genta, and lowland rice genotype Taichung 65 showed the higher degree of osmotic adjustment than that of upland genotype 4621. Upland rice genotypes with high degree of osmotic adjustment showed high values of leaf conductance, net photosynthetic rate, leaf area and dry matter under long-term condition with severe water deficit, and also showed high values of leaf conductance and net photosynthetic rate under short-term water deficit. It appeared that under long-term with severe water deficit, the leaf area is more important than the photosynthetic rate in determining the dry matter production. Besides, under short-term water deficit, the lowland genotype, Taichung 65, showed an osmotic adjustment as high as those in upland genotypes 4652 and Genta, but it could not maintain its photosynthetic rate and leaf conductance as high as 4652 and Genta when soil water potential decreased.

Introduction

Stomatal opening and leaf area expansion decrease under water deficit (Begg and Turner, 1976; Cutler *et al.*, 1980; O'Toole and Cruz, 1980). These responses may reduce transpiration, but it also reduces photosynthetic capacity and dry matter production. Osmotic adjustment, however, helps in maintaining turgor, and hence stomatal opening (Henson *et al.*, 1983; Hsiao *et al.*, 1984) and leaf elongation (Cutler *et al.*, 1980), as well as delaying leaf senescence (Hsiao *et al.*, 1984). Furthermore, osmotic adjustment helps to maintain root development (Wright and Smith, 1983), allowing the plant to absorb water to continue photosynthetic activity over a wider range of soil water potentials. Accumulated evidences (Johnson *et al.*, 1984; Jones, 1980; Ludlow, 1980; Nicolas *et al.*, 1985; Wright *et al.*, 1983) suggested that this type of plant could be advantageous in an environment with highly variable rainfall.

Rice is cultivated mainly in the monsoon areas of Asia. Several workers have reported that the stomata of upland rice are more sensitive to water deficit than those of lowland rice genotypes (O'Toole and Cruz, 1980; Singh and Sasahara, 1981; Yang *et al.*, 1983), and that the degree of osmotic adjustment of upland rice under water deficit was less than that in lowland rice (Hsiao *et al.*, 1984; Turner *et al.*, 1986; Yang *et al.*, 1983). These results indicated that osmotic adjustment is disadvantageous to drought tolerence in rice plants, which were inconsistent with the results in other crops (Johnson *et al.*, 1984; Nicolas *et al.*, 1985; Wright *et al.*, 1983). Probably, most of those previous studies only used a limited number of genotypes in a single drought condition, therefore, the collected information might not be widely

generalized. In the present study different genotypes were used to determine the genotypical difference in osmotic adjustment under gradually and rapidly developing water deficits. The effects of osmotic adjustment on photosynthesis and dry matter production were also investigated.

Materials and methods

Three genotypes of upland rice, 4621, 4652 and Genta, and a lowland genotype, Taichung 65 (Table 1) were used for experiments with both long-term and short-term water deficit stresses.

In the long-term water deficit experiment, seeds were sown in plastic trays with 4 cm depth of soil. The soil in the trays was partitioned into 3 cm × 3 cm blocks with thick paper, and three seeds were sown in each block. At the 4-leaf stage, the three seedlings with soil block were transplanted into a plastic pot (21.5 cm diameter, 17 cm deep) filled with soil. Fertilizers were applied at 0.5-0.5-0.5 g N-P-K per pot. The pots were irrigated with enough water daily to keep the soil near field capacity until 10 days after transplanting. Then, an autoirrigation apparatus with polyvinyl formal (PVF) sponge (Kanebo Ltd Japan) was used to control the soil water status in the pots. A PVF sponge, 3 cm wide and 3 mm thick, was placed through the soil down to a water bottle. Water was absorbed by PVF and exuded into soil in the pot. Soil water potential in the pots was controlled by adjusting the PVF length. The lengths of PVF sponges (from water surface to pot bottom) used were 1, 35 and 65 cm, and soil water potentials 2 cm away from PVF were about −0.25, −1 and −2.5 MPa, respectively. All measurements were taken at 40—50 days after transplanting.

For testing the short-term water deficit, seedlings at the 4-leaf stage were transplanted into 1/5000 a Wagner pots (16 cm diameter, 19.5 cm deep) filled with soil, with a hill of 3 seedlings per pot. Fertilizers were applied at 0.5-0.5-0.5 g N-P-K per pot. Pots were flooded until 40—50 days after transplanting, from this time, watering was stopped, all the measurements were carried after water was withheld for 5—7 days, at which time the photosynthetic rate was reduced to about 30% of that in well-watered conditions.

Table 1. Rice varieties used

Genotype	Origin
4621[a]	Taiwan mountainous upland genotype
4652	Taiwan mountainous upland genotype
Genta	San Salvador upland genotype, Indica (IR841 × C46-151)
Taichung 65	Taiwan lowland genotype, Japonica (Kameji × Shinriki)

[a] Acc. No., preserved in Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan.

Net photosynthetic rate and transpiration rate were determined on intact, uppermost fully-expanded leaves in an open gas exchange system at midday. Rates of CO_2 and H_2O exchange were determined with an infrared gas analyzer (Anarad, AR-600) at 30°C, 80% RH, 1000 $\mu E\,m^{-2}\,sec^{-1}$ photon flux density (PAR), and 1.5 $m\,sec^{-1}$ wind speed as described by Weng and Chen (1987). Leaf conductance was calculated from vapor pressure deficits and transpiration rate of the leaves (Gaastra, 1959).

Leaf and soil water potentials were estimated with a thermocouple psychrometer (Wescor C52 sample chambers connected to a Wescor HR33 dewpoint microvolt meter), at 8.30, 11.00 and 14.30 h (local time). Leaf osmotic potential was obtained by freezing leaf tissue in liquid N_2 and thawing at room temperature prior to equilibration in a thermocouple psychrometer. Turgor potential was calculated from leaf water potential and osmotic potential by their difference. The uppermost fully-expanded leaf was used to measure leaf water potential and osmotic potential.

Results

The relationship between leaf water potential and leaf osmotic potential in four rice genotypes under long-term water deficits are shown in Fig. 1. In all genotypes, the leaf osmotic potential decreased linearly as leaf water potential decreased. However, the slope of the regression line between leaf water potential and osmotic potential varied among genotypes. Upland genotype 4621 showed the highest slope, followed by 4652, Genta, and lowland genotype Taichung 65. This result indicates that the degree of osmotic adjustment can be ranked from high to low as 4621, 4652, Genta and Taichung 65. Similar ranking was also ob-

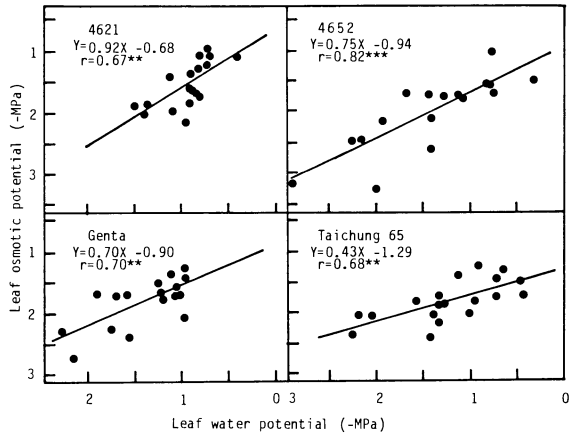

Fig. 1. Relationship between leaf osmotic potential and leaf water potential under long-term water deficit. Data were obtained by measuring from three levels of soil water potentials at 8.30, 11.00 and 14.30 h. **, ***, significant at 0.01 and 0.001 levels, respectively.

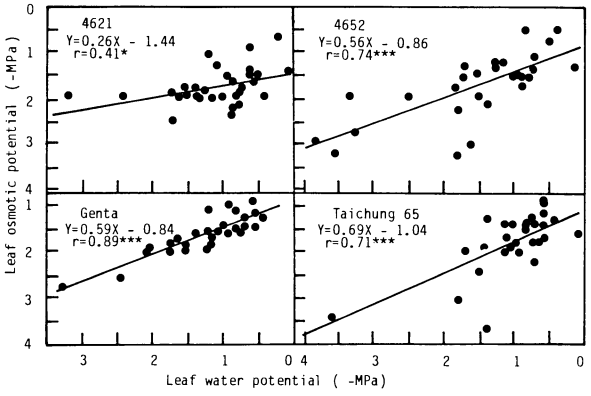

Fig. 2. Relationship between leaf osmotic potential and leaf water potential under short-term water deficit, *, ***, significant at 0.05 and 0.001 levels, respectively.

served for the decreasing rates of turgor potentials of these genotypes under water deficit (Table 2).

Dry matter production, leaf area, and net photosynthetic rate of single leaves (Pn) decreased as soil water potential decreased (Table 2). Under a medium water deficit (PVF length 35 cm), dry matter production, leaf area, and Pn decreased about 40—50%, 50—60% and 20—30% of wet condition, respectively, and those under a severe water deficit (PVF length 65 cm), they decreased about 70—85%, 80—95% and 50—80% respectively. However, the genotypes with a higher degree of osmotic adjustment could maintain higher leaf turgor potentials under medium and severe water deficit and showed higher dry weight, leaf area, Pn and leaf conductance, under severe water deficit, than the genotypes with lower osmotic adjustment. These results indicate that osmotic adjustment is an adapting mechanism for drought tolerance as it helps the rice plants to maintain leaf turgor, leaf area and Pn, and to promote dry matter production.

The degree of inhibition in leaf area under long-term water deficit was higher than that of Pn (Table

Table 2. Dry matter production and some leaf traits of rice plants grown under three levels of soil water potentials

Genotype	Treatment[a]	Dry weight ($g\,pot^{-1}$)	Leaf area ($cm\,pot^{-1}$)	Photosynthetic rate ($mg\,CO_2\,dm^{-2}\,h^{-1}$)	Leaf conductance ($cm\,s^{-1}$)	Leaf turgor[b] potential (MPa)
4621	A	10.34 ± 1.11(100)	923 ± 200(100)	29.30 ± 2.32(100)	0.30 ± 0.05(100)	0.53
	B	5.78 ± 1.63 (56)	462 ± 108 (50)	20.66 ± 1.79 (70)	0.23 ± 0.03 (61)	0.85
	C	3.20 ± 0.75 (31)	203 ± 10 (22)	14.40 ± 1.29 (49)	0.08 ± 0.02 (21)	0.44
4652	A	15.24 ± 1.58(100)	1353 ± 209(100)	23.88 ± 0.67(100)	0.29 ± 0.04(100)	0.50
	B	8.92 ± 1.08 (59)	609 ± 124 (45)	19.00 ± 3.13 (80)	0.21 ± 0.02 (72)	0.72
	C	2.44 ± 0.90 (16)	116 ± 66 (9)	8.77 ± 0.07 (37)	0.06 ± 0.01 (21)	0.24
Genta	A	13.89 ± 2.25(100)	1389 ± 241(100)	28.57 ± 0.41(100)	0.34 ± 0.06(100)	0.55
	B	6.39 ± 2.44 (46)	575 ± 220 (41)	22.64 ± 2.83 (79)	0.21 ± 0.09 (62)	0.34
	C	2.01 ± 0.17 (14)	127 ± 11 (9)	12.13 ± 2.57 (42)	0.07 ± 0.01 (20)	0.31
Taichung 65	A	9.13 ± 0.56(100)	735 ± 96 (100)	28.31 ± 3.18(100)	0.40 ± 0.07(100)	0.61
	B	4.94 ± 0.56 (54)	345 ± 38 (47)	21.19 ± 2.01 (75)	0.24 ± 0.06 (60)	0.59
	C	1.17 ± 0.07 (13)	52 ± 3 (7)	5.04 ± 2.85 (18)	0.03 ± 0.01 (7)	0.02

[a] Potentials 2 cm away from PVF were about −0.25, −1 and −2.5 MPa, respectively.
[b] Average of measurements at 8.30, 11.00 and 14.30 h.

2). The correlation coefficient between dry weight and leaf area was as high as 0.99 (n = 12) and that between dry weight and Pn was 0.82 (n = 12). This suggests that the decrease of leaf area is more important than that in Pn in determining dry matter production under long-term water deficit.

In response to a short-term water deficit, leaf osmotic potentials decreased linearly with decrease in leaf water potential in all genotypes (Fig. 2). However, the slopes of the regression lines between leaf water potential and osmotic potential were lower than those under long-term water deficit. This result indicates that osmotic adjustment was less for short-term water deficit than for long-term water deficit. In Fig. 1, the upland genotype 4621 showed a higher degree of osmotic adjustment than other genotypes under the long-term water deficit. Nevertheless, the lowest degree of osmotic adjustment was observed in 4621 as compared with the other three genotypes, under short-term water deficit. On the other hand, Taichung 65, which was a lowland genotype showed the lowest degree of osmotic adjustment under long-term water deficit, but maintained the higher osmotic adjustment, comparable to upland genotypes 4652 and Genta under short-term water deficit.

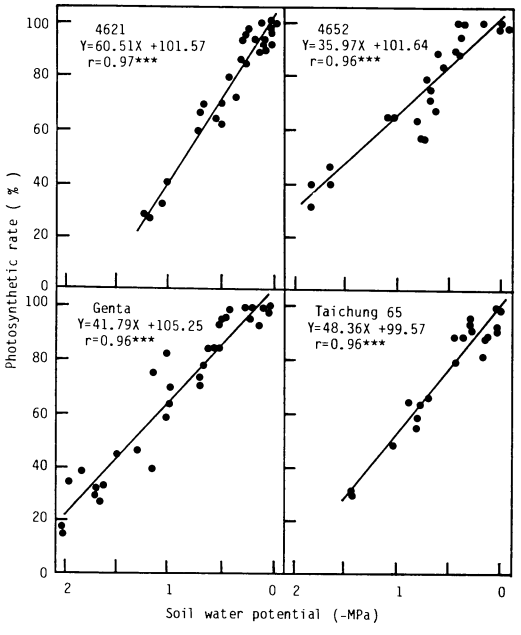

Fig. 3. Relationship between leaf net photosynthetic rate and soil water potential under short-term water deficit. ***, significant at 0.001 level.

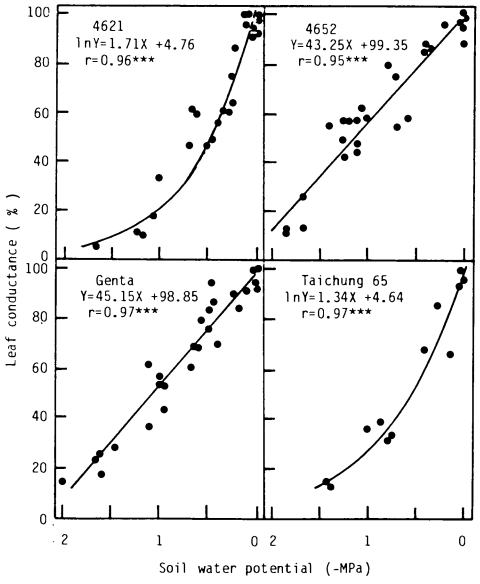

Fig. 4. Relationship between leaf conductance and soil water potential under short-term water deficit. ***, significant at 0.001 level.

The Pn and leaf conductance decreased as soil water potential decreased (Fig. 3; Fig. 4). Photosynthetic rate declined linearly with the decrease in soil water potential. A linear decline in leaf conductance was also observed in genotypes 4652 and Genta as soil water potential decreased from -0.1 MPa to -2 MPa, but a curvelinear response in leaf conductance was observed in genotypes 4621 and Taichung 65. Genotypes 4652 and Genta showed higher degree of osmotic adjustment and lower degree of inhibition on Pn and leaf conductance than genotype 4621. These results suggest that osmotic adjustment was advantageous to Pn and leaf conductance under water deficit conditions. However, the lowland genotype, Taichung 65, showed an osmotic adjustment as high as those in upland genotypes 4652 and Genta, but it could not maintain its Pn and leaf conductance as high as 4652 and Genta when soil water decreased.

Discussion

The present data indicate that genotypical differences in osmotic adjustment indeed exist in rice plants and varied with the development of water

deficit. In long-term water deficit treatment began 10 days after transplanting, at that time plants were small, had only 20 — 30 cm^2 leaf area per pot, could use small amounts of water, so stress developed slowly. Under this condition the three upland rice genotypes showed the highest degrees of osmotic adjustment. However, in the short-term water deficit, which was initiated at 40 to 50 days after transplanting, the plants were grown to have 800 — 1300 cm^2 leaf area per pot, and used more water, so the stress was imposed rapidly, upland rice genotypes 4652 and Genta and the lowland rice genotype Taichung 65 showed a higher degree of osmotic adjustment than the upland genotype 4621.

Osmotic adjustment of leaf tissues has been reported to be capable of maintaining leaf conductance (Hsiao *et al.*, 1984; Yang *et al.*, 1983) and leaf elongation (Cutler *et al.*, 1980) in rice plants under water deficit. Similar conclusions were also drawn in the present study, in which the upland rice genotypes with higher osmotic adjustment capacity tended to have higher leaf conductance, Pn, leaf area and dry matter accumulation under water deficit. These results indicate that osmotic adjustment indeed plays an important role in regulating drought tolerance in rice plants. Similar results have also been shown in other crops, such as sorghum and wheat (Johnson *et al.*, 1984; Nicolas *et al.*, 1985; Wright *et al.*, 1983).

Many previous reports (Hsiao *et al.*, 1984; O'Toole and Cruz, 1980; Singh and Sasahara, 1981; Turner *et al.*, 1986; Yang *et al.*, 1983) have come to the conclusion that the upland rice had a lower degree of osmotic adjustment than lowland rice. They further confirmed that the stomatal response to water deficit in upland rice was more sensitive as compared with lowland rice. However, the results presented herein did not support their findings. Genotypical differences might be able to partially elucidate the contrary results. The data in Fig. 1 and Fig. 2 have shown that the pattern of osmotic adjustment varied with the timing of stress development. Besides, the lowland genotype Taichung 65 which capable of maintaining osmotic adjustment as high as that of the upland genotypes 4652 and Genta, was unable to keep its Pn and leaf conductance at the level equivalent to that of 4652 and Genta when soil water potential decreased. This result indicates that drought tolerance of the rice plant would also be influenced by other different characteristics (O'Tool and Chang, 1979; Turner *et al.*, 1986).

Chang *et al.* (1972) found upland-adapted rices to possess deeper root systems than lowland genotypes. O'Toole *et al.* (1978) showed that many upland genotypes were poorly adapted to drought condition if the root development was limited. It might be assumed that some upland rice genotypes with drought tolerance owed that to their root system. In this study, root was limited in a small pot, Pn and dry matter production were mainly favoured by osmotic adjustment.

Acknowledgements

This research was supported by a grant from the National Science Council, ROC. I thank Prof C Y Chen, Prof C C Young, Prof F J M Sung and Dr F H Hsu for their helpful discussion and suggestions. Also thanks to Kanebo Ltd (Osaka, Japan) for providing PVF sponges.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 151–161.
PLSO PN 146
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Evaluation of breeding strategies for drought tolerance in potato by means of crop growth simulation

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Key words: breeding, drought tolerance, potato, simulation model

Abstract

Breeding strategies for drought tolerance in potato were evaluated by means of a crop growth model, in which seasonal courses of crop dry matter accumulation and soil moisture availability were simulated in dependence of plant characteristics and weather and soil data.

Several plant characteristics substantially influenced the simulated instantaneous water consumption of the genotype. However, effects of genotypic differences on final tuber yield were much smaller because of the close relationship between transpiration and growth. Hence, a lower water consumption not only saved water for later use, but was also at the expense of the actual growth rate. Selection for low-transpiration types, at unchanged water use efficiency, would result in lower yields under optimum conditions.

Short periods of drought, in general, reduced tuber yield of late genotypes less than that of early genotypes. Late genotypes had a surplus of leaf area for full light interception giving a lower impact of leaf area reduction. Late drought affected early genotypes less because of escape.

The simulation results emphasized the complexity of selection for drought tolerance caused by the many plant processes involved, the contrast between instantaneous and cumulative reactions and the strong genotype × environment interaction for drought tolerance.

Introduction

Potato yields are substantially reduced by drought. Cultivar differences in drought tolerance have been demonstrated by Harris (1978), Steckel and Gray (1979), Levy (1983b; 1986), Susnoschi and Shimshi (1985), van Loon (1986), and Jefferies and MacKerron (1987). However, selection for drought tolerance is complicated by the fact that the differences in yield reduction cannot be traced back to one or a few major morphological or physiological components, for which an efficient screening technique could be developed. Moreover, genotypic differences in plant responses to drought may vary from year to year and from site to site (genotype × environment interaction). These difficulties are due to the fact that the ability to maintain a high yield under drought is determined by many characteristics, and the importance of each factor varies with time and severity of the stress.

Crop growth models facilitate the extrapolation of effects on the level of single plant organs to the growth of a complete canopy over the season in a continuously changing field environment. In the present paper, crop growth simulation is used as a tool to gain more insight into the morpho-physiological components of drought tolerance in potato.

Various approaches have been developed for modelling crop growth in response to drought. Also, several crop growth models have been published for potato, differing in approach and detail (Fishman *et al.*, 1985; Haverkort and Harris, 1987; Ingram and McCloud, 1984; Johnson *et al.*, 1986; MacKerron *et al.*, 1985; Ng and Loomis, 1984; Sands *et al.*, 1979; Spitters, 1987; Spitters *et*

al., 1989). The model presented here is simple, but sufficiently detailed to evaluate the influence of various components of drought tolerance in relation to the environment.

Model description

The model is depicted in Figure 1 and described in detail in the Appendix. Total dry matter production is calculated from the incident amount of photosynthetically active radiation (PAR, 400—700 nm), the fraction of PAR intercepted by the crop — being a function of its leaf area index — and the light utilization efficiency. Tuber yield is determined by total dry matter growth and the fraction of dry matter allocated to the tubers.

Crop growth under optimal soil moisture supply

The calculation of crop growth under adequate soil moisture conditions is based on the general crop growth simulator presented by Spitters *et al.* (1989), except that the detailed procedures for photosynthesis and respiration have been replaced by a single parameter characterizing the average efficiency of light utilization (ε in g DM/MJ PAR). Allowance is made for the lower efficiency of conversion of assimilates into structural dry matter for haulms compared to tubers (0.68 and 0.78 g DM/g CH_2O for haulms and tubers, respectively; Penning de Vries *et al.*, 1983). The parameters and functions specific for potato were mainly derived from experiments carried out in 1985 and 1986 in the Dutch Flevopolder where various cultivars were harvested at intervals (Spitters, 1987; Spitters and Neele, in prep.). Dry matter partitioning and leaf area dynamics are related to the maturity class (m) of the cultivar.

Soil moisture balance

A simple moisture balance for a free draining soil profile is incorporated in the model. This balance is mainly borrowed from van Keulen (1975; 1986; Spitters, 1989). The daily change in soil moisture content in the rootable zone is calculated from rainfall, crop transpiration, soil evaporation and deep percolation (Fig. 1). Percolation is calculated as the amount of water in excess of field capacity,

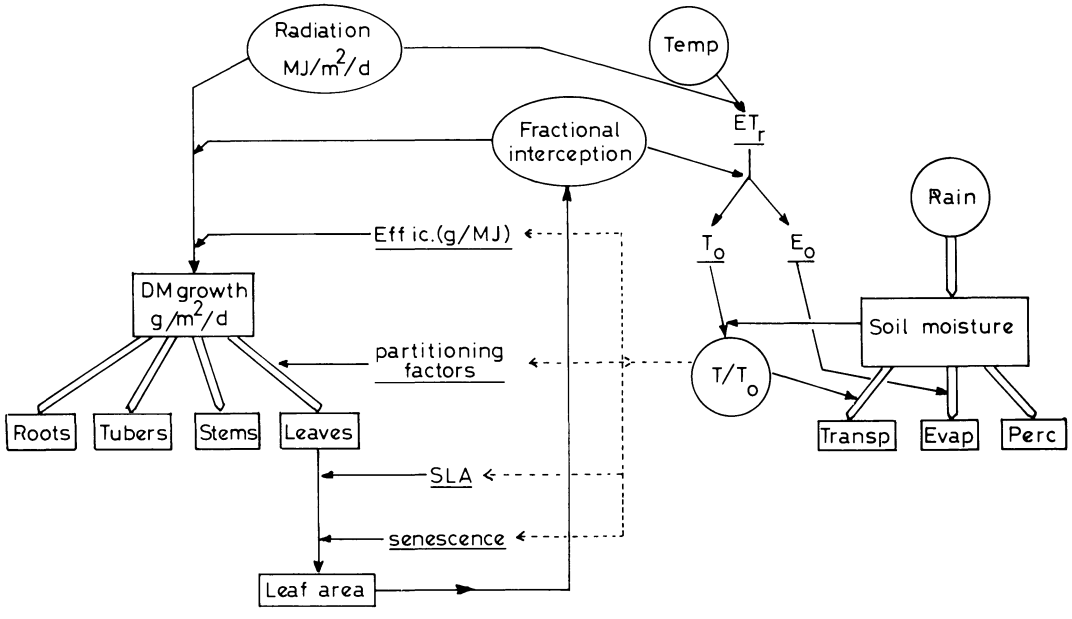

Fig. 1. Simplified scheme for the calculation of daily crop growth in relation to weather data and soil moisture availability. Drought stress, quantified by the ratio of actual to potential transpiration (T/T_0), affects the light utilization efficiency, the dry matter partitioning, the specific leaf area (SLA) and the rate of leaf senescence.

which drains below the root zone. Potential transpiration and evaporation are related to the reference evapotranspiration rate of a short grass cover —calculated by the Makkink (1957; de Bruin, 1987) equation from daily radiation and temperature— and the fractional interception of solar radiation by the canopy. Soil evaporation is reduced when the upper 2-cm layer of the soil dries out; the water content of the surface layer is tracked in a separate moisture balance.

Crop-water relations

When the soil dries out, stomata close and transpiration falls below its potential value. The ratio between actual and potential transpiration (T/T_0) is supposed to decrease linearly with soil moisture suction from unity at the critical suction to zero at wilting point (Feddes *et al.*, 1978). In potato, the critical suction was found to increase from about 400 cm at an evaporative demand of 5 mm d^{-1} to 600 cm (on loamy sand) or 800 cm (on clay) at an evaporative demand of 1 mm d^{-1} (P Kabat, ICW, Wageningen, pers. commun.; Taylor and Ashcroft, 1972). Wilting point was estimated to be 8000 cm (or 16000 cm if a linear reduction with pF, rather than with cm suction, is assumed). The factor (T/T_0) to reduce transpiration is also applied to the rate of crop growth.

Drought often affects the distribution of assimilates between above- and below-ground parts in favour of the latter (Munns and Pearson, 1974). It may also reduce the specific leaf area, and mostly it accelerates leaf senescence (Ludlow, 1975). The description of the effect of water stress on the relative death rate of the green leaf area is borrowed from van Keulen (1986). Leaf longevity is in general not reduced by early drought (van Loon, 1981; Vos, 1987) so that in the model the acceleration of leaf senescence is only operative from the beginning of the normal, developmental ageing. The applied reduction in specific leaf area of leaves developing under dry conditions is based on the range in values of specific leaf area encountered in field experiments. Possible effects of specific leaf area on life span and photosynthetic capacity of the leaves were neglected. The effect of drought on the partitioning of current assimilates between haulms and below-ground parts is described according to van Keulen *et al.* (1981).

Input data

Input for the model are standard data of daily solar radiation, temperature and rainfall; plant density, dates of crop emergence and harvest; rootable depth; and the soil moisture retention characteristics, *i.e.* the relation between volumetric soil moisture content and suction.

Model validation and sensitivity analysis

Model validation

Separate elements of the model were based on other crop growth models and have been previously validated (*e.g.*. Spitters, 1987; Spitters *et al.*, 1989; van Keulen, 1975). The section on crop growth under adequate water supply was parameterized on the basis of two field experiments (Spitters, 1987; Spitters and Neele, in prep.) and thus calibrated on those conditions. The reduction of crop transpiration in relation to soil moisture suction was estimated from various soil moisture balance studies with potato in the Netherlands (Feddes *et al.*, 1988; P Kabat, ICW, Wageningen, pers. commun.). On the other hand, the response of assimilate partitioning, specific leaf area and leaf senescence to the degree of water stress still requires better parameterization since quantitative data were scarce or absent.

Sensitivity analysis

The influence of various plant characteristics on the drought tolerance of a cultivar was evaluated by changing these characteristics in a reference version of the model and by studying the response of tuber yield with and without adequate water supply.

In the reference version of the model, 30-year averages of solar radiation and temperature for Wageningen, The Netherlands, were used to describe the seasonal course of the weather. The crop-specific parameters are given in the Appendix, and simulations were performed for a mid-early variety (m = 7) and a mid-late variety (m = 4). Crop emergence occurs on 15 May at 38,000 plants ha^{-1} and the crop is harvested on 17 September. The soil is a light-loam sand, having a

volumetric moisture content of 23.4% at field capacity (pF 2.3) and 5.3% at pF 4.2 and a rootable depth of 0.40 m.

The influence of various plant attributes on the drought response of the genotype was evaluated for a stress lasting over a long period of time (Fig. 2a, Tables 1 and 2). Soil moisture content was initialized on 20 April with the value at field capacity, and daily rainfall was supposed to be 70% of the reference evapotranspiration of a short grass cover. Because of the use of average rainfall data, soil evaporation was reduced here in dependence of the average soil moisture content of the whole rootable zone instead of that of the surface layer only, as the latter approach requires daily rainfall data as input (Spitters, 1989).

Not only the effects of sustained stress, but also the influence of a single short period of drought was evaluated, in relation to the time of its occurrence and the maturity class of the cultivar. Transpiration was set to decline according to a fixed pattern (Fig. 2b), which was the same for the different drought periods during the season (Fig. 3).

Results and discussion

General effects of water stress

The effects of water shortage on growth and tuber yield, as accounted for in the model, are (Fig. 1): (a) Reduction in dry matter increment per unit of intercepted light (ε in g/MJ), (b) reduction in degree and duration of light interception due to (b1) less leaf area growth when the stress occurs early in the season. The reduction in leaf area growth is caused by a lower rate of photosynthesis, a decrease in specific leaf area, and a lower proportion of the assimilates allocated to haulm growth. (b2) Accelerated leaf senescence when the stress occurs in the second half of the growing period. (c) A slight increase in the proportion of assimilates allocated to tuber growth when the stress occurs during tuber bulking.

Effects of drought on tuber quality and grading (van Loon, 1986), and decreased lodging susceptibility of the crop have not been taken into account.

Time of transient drought

The simulated effects of transient drought on tuber yield were, on average, strongest for early cultivars (Fig. 3). Due to early tuber bulking, these varieties produce less leaf area (Fig. 4b) and show earlier foliage senescence resulting in a smaller cumulative light interception (Fig. 4a). Stress during the stage of leaf area expansion—which reduces leaf production—therefore had a greater impact on early varieties than on late varieties which have a surplus of leaf area for full light interception. For similar reasons, stress during the early stage of foliage senescence had a greater effect on early than on late varieties. However, stress late

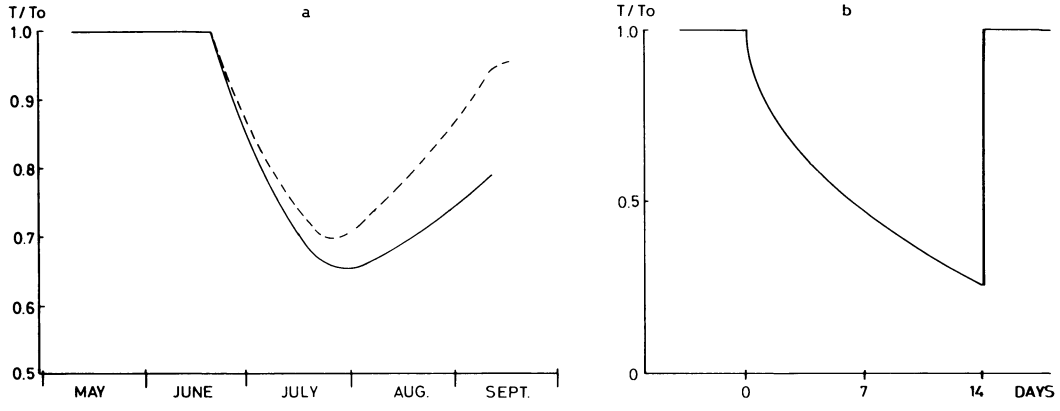

Fig. 2. **(a)** Simulated seasonal course of the ratio of actual to potential transpiration for a mid-early (*broken curve*) and a mid-late cultivar (*solid curve*) in the case of long-term stress. **(b)** Time course of the ratio of actual to potential transpiration applied to evaluate the effect of short periods of drought.

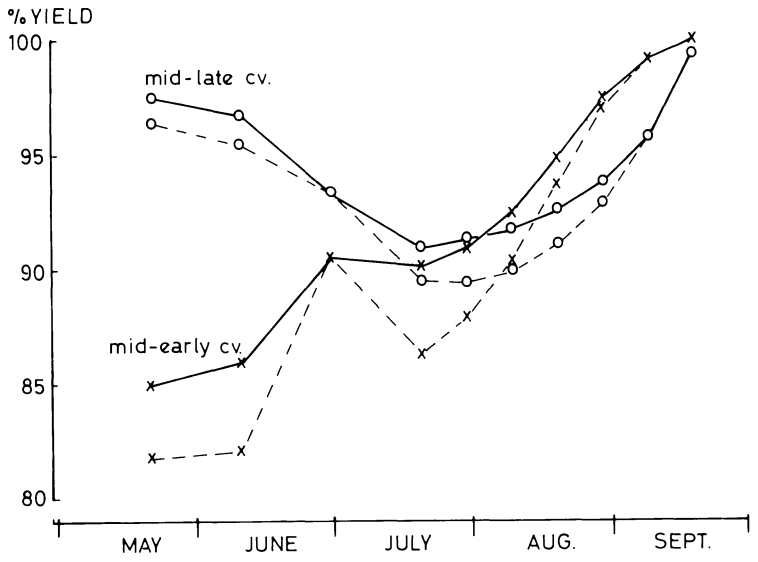

Fig. 3. Simulated effects of transient drought on tuber yield of a mid-early (x) and a mid-late (O) potato cultivar in relation to time of occurrence of the stress. *Each data point* refers to the mid point of a 14-day stress period. *Solid curves* refer to a reduction in light utilization only, and *broken curves* refer to simulations with effects on dry matter partitioning, specific leaf area and leaf senescence included. Yield is expressed relative to the tuber yield under optimal water supply, being 13.0 and 14.7 ton DM ha^{-1} for the early and the late cultivar, respectively.

in the season affected late cultivars most, whereas early cultivars partly escaped from this type of drought since they had almost completed their growth cycle at that time (Fig. 4a).

The effect of timing of the stress on tuber yield was divided into the effects on cumulative light interception, seasonal light use efficiency, and harvest index (Fig. 5). In the simulations, cumulative light interception was reduced most by stress during the phase of leaf area expansion, especially in early cultivars. A certain percentage of reduction in growth rate had its greatest influence on the seasonal light use efficiency when it occurred during a phase of rapid growth, *i.e.* during full ground cover and high incoming solar radiation (Fig. 4 and 5). In the early phases, tuber growth is still absent or small so that drought reduced haulm growth more than tuber growth, increasing the harvest

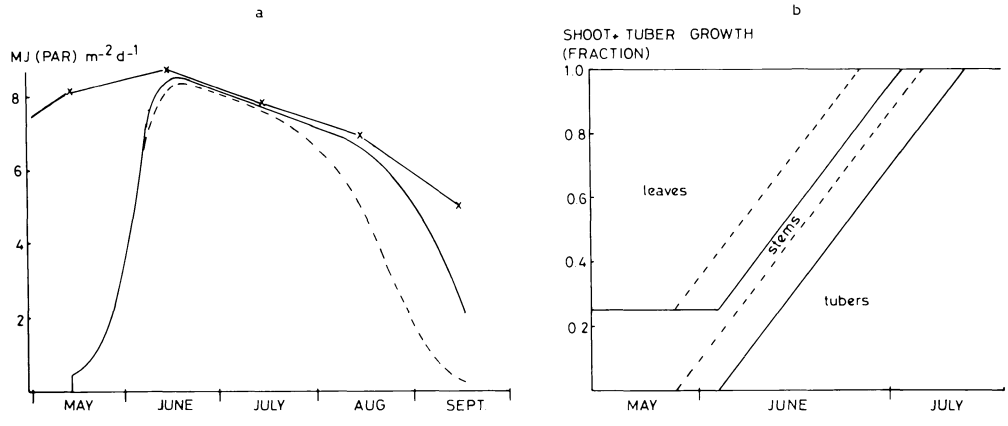

Fig. 4. **(a)** Incoming photosynthetically active radiation (x, 30-year average for Wageningen) and simulated light interception for a mid-early (*broken curve*) and mid-late variety (*solid curve*) under adequate water supply. **(b)** Simulated fraction of current dry matter growth partitioned to leaves, stems and tubers for a mid-early (*broken curves*) and a mid-late variety (*solid curves*).

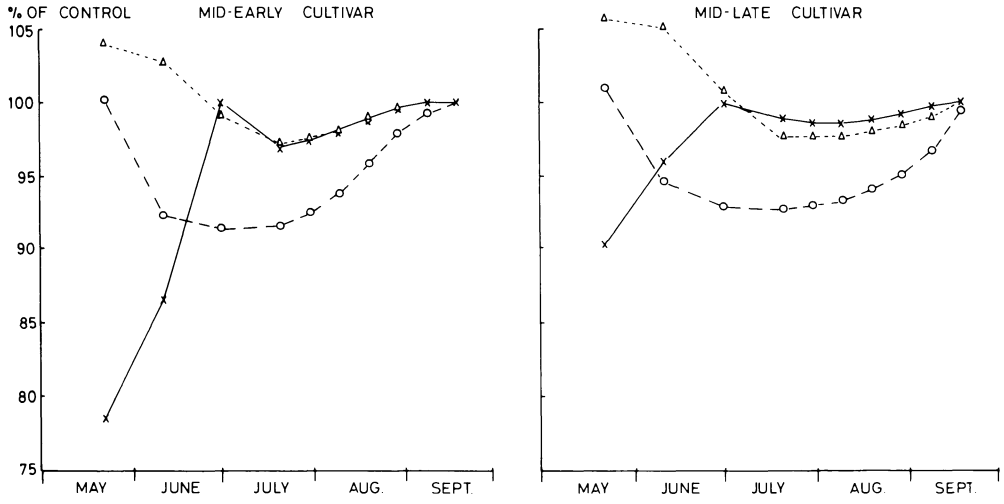

Fig. 5. Simulated effect of transient drought on cumulative light interception (x, Σ PAR), seasonal averaged light use efficiency (○, $\bar{\varepsilon}$) and harvest index (Δ, HI) for a mid-early and a mid-late potato variety ($\bar{\varepsilon}$ and HI with fibrous roots excluded). *Each data point* refers to the middle of the 14-day stress period. Simulations included the effects of drought on dry matter partitioning, SLA and leaf senescence. Values are expressed relative to those under optimal water supply: Σ PAR = 629 and 747 MJ m^{-2}, $\bar{\varepsilon}$ = 2.52 and 2.49 g DM MJ^{-1} and HI = 0.82 and 0.79 for the mid-early and mid-late cultivar, respectively.

index. Drought during the phase of declining tuber bulking rates evidently reduced harvest index less than when it occurred during the phase of rapid tuber bulking (Fig. 5).

Finally there is an effect of scales considering percentage of yield reduction. The same absolute yield reduction (in kg ha^{-1}) means at a lower yield level a greater percentage of reduction; simulated tuber yields under ample water supply amounted to 13.0 and 14.7 t DM ha^{-1} for the mid-early and mid-late variety, respectively. Moreover, the same intensity and duration of reduction in tuber bulking rate gives a greater percentage of yield reduction with a short than with a long tuber filling period.

Effect of plant characteristics

The influence of various plant attributes on the drought tolerance of a genotype was evaluated for the situation where the stress extended over a sustained period (Fig. 2a; Tables 1 and 2).

Water use efficiency. If we could lower the rate of transpiration without affecting the potential rate of growth, the water use efficiency — the dry matter production per unit of transpired water — would raise and the yield under drought stress increase (Table 1, line 2).

Transpiration and photosynthesis are, however,

Table 1. Simulated effect of 10% changes in transpiration (T) and water use efficiency (WUE) on tuber yield (t DM ha^{-1}) for a mid-early and a mid-late cultivar under optimal conditions (0) and under two different conditions of drought stress (1 and 2). Stress condition 2 is depicted in Figure 2a for the reference situation. Condition 1 was the same, except that the stress lasted till 1 August only. The multiplication factor was used to alter the specified attribute in the model with respect to the reference. Between brackets, tuber yields expressed as a percentage of the non-stressed control

	Multiplication factor		Mid-early cultivar			Mid-late cultivar		
	Growth	Transp.	0[a]	1	2	0	1	2
1. Reference	1.0	1.0	13.0	10.9(84)	9.9(76)	14.7	12.7(86)	10.4(71)
2. T↓, WUE↑	1.0	0.9	13.0	11.8(91)	11.0(85)	14.7	13.6(92)	11.8(80)
3. T↓, WUE constant	0.9	0.9	11.4	10.4(91)	9.7(86)	13.2	12.2(92)	10.6(80)
4. T constant, WUE↑	1.1	1.0	14.5	12.1(83)	10.9(75)	16.2	14.0(86)	11.4(71)

[a] 0, optimal conditions; 1, drought stress condition 1 and 2, drought stress condition 2.

Table 2. Simulated responses of variation in plant attributes on tuber yield (t DM ha^{-1}) for a mid-early and a mid-late cultivar under ample water supply and under water stress. The multiplication factor was used to alter the specified parameters in the model with respect to the reference

	Multipl. factor	Mid-early cv.		Mid-late cv.	
		Control	Stress	Control	Stress
1. Reference		12.98	9.87	14.70	10.41
2. Critical suction	1.5	12.98	9.94	14.70	10.43
3. Wilting point	1.5	12.98	10.20	14.70	10.68
4. Leaf area expansion rate	1.2	13.57	9.90	14.81	10.28
5. D.m. distribution pattern[a]		12.74	9.73	15.03	10.83
6. Drought effect on leaf senescence	0.8	12.98	9.96	14.70	10.49

[a] D.m. distribution pattern (Fig. 4b): onset of tuber filling × 0.6 and slope of allocation to tubers × 0.8.

often closely linked so that a reduction in transpiration rate usually involves a similar reduction in growth rate and the water use efficiency remains constant. Though low-transpiration types had a smaller relative yield reduction in dry conditions, their yields under optimal water supply were smaller and, depending on the severity of the stress, also their absolute yields under dry conditions (Table 1, line 3). This is a warning against unconscious selection for low water consumption.

Increasing water use efficiency at constant potential transpiration rate gave the highest yield under conditions of ample water supply or moderate stress (Table 1, line 4). Under severe drought, however, genotypes which combine a high water use efficiency with a low transpiration rate may produce the higher yields because of a better distribution of the available moisture over the season (Table 1, line 2 *vs.* line 4). This illustrates that time and severity of the stress determine whether either saving water or spending water would be the best strategy for obtaining high yields.

The strong correlation between transpiration and photosynthesis is reflected in stomatal regulation such that the CO_2 concentration inside the leaf remains approximately constant (Wong *et al.*, 1979). Increasing the water use efficiency would thus require a reduction of internal CO_2 concentration. This could be selected for on the basis of the $^{13}C/^{12}C$ carbon isotope ratio of the plant (Farquhar *et al.*, 1982). Genetic variation in this ratio and in the associated variation in water use efficiency has been demonstrated for several species, among them potato (Farquhar and Richards, 1984; Hubick *et al.*, 1986; Vos and Groenwold, 1989). Selection for lower internal CO_2 concentration has however the risk of selecting genotypes showing a reduced photosynthetic rate under optimal water supply, since the rate of photosynthesis is a function of the internal CO_2 concentration.

Critical suction. Varietal differences have been demonstrated in timing of stomatal closure when potato plants are subjected to water stress (Levy, 1983a; Schapendonk *et al.*, 1988; van Loon, 1981). In the model, such differences are reflected in the critical soil moisture suction above which the transpiration is reduced. Increasing this suction by 50%, *i.e.* from 500 cm (pF 2.7) to 750 cm (pF 2.9) at an evaporative demand of 3 mm d^{-1}, had little effect on tuber yield (Table 2, line 2). The substantial difference in the instantaneous reaction to water stress was leveled off when the effects were integrated over the whole season. That was because early stomatal closure, associated with a lower critical suction, saved water for later use, but at the expense of actual growth. *Vice versa*, a retarded stomatal closure let continue the potential growth rate for a longer period of time, but at the expense of the soil moisture reserves and thus at the expense of later growth. This is the dilemma involved in requiring open stomata for photosynthesis and closed stomata for minimum water loss.

In the present paper the effects were evaluated for a temperate climate. The critical suction is lower in semi-arid climates because of the higher evaporative demand, a direct effect of low relative humidity on stomatal opening (Schulze, 1986) and the mutually reinforcing effects of drought and high temperature on photosynthesis (Schapendonk *et al.*, 1989). Under such extreme conditions, genetic variation for drought tolerance might be greater. Moreover, maintaining open stomata likely has the risk of desiccation and damage of leaf

tissue; the probability being greater under more severe drought.

Genetic differences have also been shown for time and degree of wilting of plants subjected to drought in pots (Beekman and Bouma, 1986). Shifting the wilting point in the simulations to a higher level yielded some benefit (Table 2, line 3).

Leaf area. Magnifying the leaf area expansion rate at unchanged dry matter distribution increased the yield under optimal conditions. Under drought, however, that effect was counterbalanced by the increase in transpiration due to the greater LAI (Table 2, line 4). For example, in the stress environment, the peak leaf area index of the early variety increased from a value of 3.9 to 5.3, resulting in a 7% greater cumulative light interception. However, the enhanced transpiration brought about by the higher LAI resulted in a more rapid depletion of soil moisture and therefore in a reduced average light use efficiency and lower harvest index.

In field experiments, cv. Désirée showed a dry matter distribution pattern that deviated substantially from that defined in the model (Appendix, Fig. 2b; Spitters, 1987). Tuber filling started earlier than in cultivars of the same maturity group, but during tuber filling a greater proportion of the assimilates were invested in leaf growth. Hence, Désirée combined an early tuber yield with sustained leaf growth. In the simulations, such an alternative distribution pattern gave a lower relative yield reduction compared with the respective controls (Table 2, line 5). In the mid-early maturity group, its yield potential was slightly inferior giving lower absolute yields both under optimal and stress conditions.

Varietal differences have been demonstrated for leaf yellowing due to water stress (Schapendonk *et al.*, 1989) and for the rate of recovery of plants after water stress (Beekman and Bouma, 1986). In the simulations, a reduction of the effect of drought on leaf senescence diminished the yield reduction (Table 2, line 6). The importance of this attribute for drought tolerance needs further research because quantitative information on the response of leaf senescence to water stress is still scanty.

Rooting pattern. The rooting density probably does not impose a restriction on water uptake, except when it falls below a relatively low value of 1 cm root length per cm^3 of soil volume (Gregory and Brown, 1987; van Noordwijk, 1983).

With a greater rooting depth, however, the crop exploits a bigger soil moisture stock. For example, the simulations started from field capacity, which mean an initial amount of 68 mm available for transpiration in the rootable profile of 0.40 m. Increasing the rootable depth from 0.40 to 0.60 m would increase the initial stock by 34 mm, being a significant contribution to the total transpiration demand of 216 and 265 mm of the early and the late cultivar, respectively.

Since potato is a relatively shallow-rooting crop with a poor ability to penetrate compacted soil layers (van Loon *et al.*, 1985), selection for deeper rooting will mainly be selection for increased rate of vertical root extension at high mechanical soil impedance.

Concluding remarks

In this paper, various aspects of drought tolerance in potato have been evaluated by means of a crop growth model. The results emphasized the complexity of selection for drought tolerance. Several plant attributes may substantially influence the instantaneous water consumption of the crop. However, when integrated over the whole season, the impact was strongly reduced and sometimes became even reversed because of the close relationship between transpiration and growth. For instance, early stomatal closure in response to drought saves water for later growth, but at the expense of actual growth. Selection for drought tolerance is further complicated by the fact that the optimal strategy to cope with drought stress varies with time and severity of the stress, which may differ from field to field and from year to year.

The complications due to the contrast between instantaneous and cumulative reactions and the strong genotype × environment interaction emphasize the need for using crop growth simulation as a tool to make the necessary extrapolations to a complete growing season and to a range of environments when different strategies of breeding for drought tolerance are analysed.

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Appendix

Description of a simulation model for daily crop growth of potato in relation to weather conditions and soil moisture availability.

Basic equations for crop growth

$\Delta W = \varepsilon \times f_{int} \times PAR \times f_{WT}$	daily crop growth rate (g DM m^{-2})
$\Delta W_j = f_j \times \Delta W$	daily growth rate of organ j (g DM m^{-2})
$f_{int} = 1 - \exp(-k \times LAI)$	fractional light interception
$\Delta LAI = \Delta LAI_g - \Delta LAI_d$	daily net increment of LAI ($m^2 m^{-2}$)
	daily leaf area growth:
$\Delta LAI_g = \Delta W_{lv} \times SLA \times f_{WL}$	if $\Sigma t_{lv} \geqslant 450^0$Cd or LAI $\geqslant 0.75$
$\Delta LAI_g = LAI \times (\exp(R_L \times (t - 2)) - 1)$	if $\Sigma t_{lv} < 450^0$Cd and LAI < 0.75. t = daily temp.
$R_L = R_{L0} \times f_{WT} \times f_{WL}$	relative leaf area growth rate ($°C^{-1} d^{-1}$)
$\Delta LAI_d = LAI \times (1 - \exp(-R))$	daily leaf area death (exponential difference eq.)
$R = R_{dv} + \text{Max}(R_{sh}, R_W)$	relative death rate of leaf area (d^{-1})

Parameters and functions for potato under optimum water supply

$\varepsilon = \varepsilon_{tb} \times (f_{tb} + (1 - f_{tb})(0.68/0.78))$	light utilization efficiency (g MJ^{-1}(PAR))
$\varepsilon_{tb} = 2.7$	light utilization efficiency during tuber bulking
$k = 1.0$	extinction coefficient for PAR
$SLA = 0.030$	leaf area/leaf dry weight ($m^2 g^{-1}$)
$R_{L0} = 0.012$	relative leaf area growth rate ($°C^{-1} d^{-1}$)
$LAI_0 = 0.0155 \times N$	LAI at crop emergence at N plants m^{-2}
$R_{sh} = 0.03$ (LAI − 4)/4 with $0 \leqslant R_{sh} \leqslant 0.03$	relative death rate (d^{-1}) due to self-shading
$R_{dv} = \Delta t_{sn} \times \exp(-11.7 + 0.68\,m) \times \exp(\Sigma t_{sn} \times (0.0068 - 0.00060\,m))$ for $\Sigma t_{lv} > 725$, with $\Sigma t_{sn} = \Sigma t_{lv} - 725$, $\Delta t_{sn} = \text{Max}(8, t - 2)$	rel. death rate (d^{-1}) due to developmental ageing as a function of temperature (t)
m	maturity group: 2.5 = very late, 9.5 = very early
$\Sigma t_{lv} = \Sigma(t - 2)$	temperature sums (^{0}Cd) after crop emergence for: leaf area dynamics
$\Sigma t_{tb} = \Sigma\,\text{Min}(t - 2, 29 - t)$ with $0 \leqslant t_{tb} \leqslant 11$	tuber growth
	fraction of d.m. growth allocated to:
$f_{shoot} = 0.80 + 0.20\,(\Sigma t_{tb} - i)/430$	$0.80 \leqslant f_{shoot} \leqslant 1.00$ shoots + tubers
$f_{lv} = 0.75 - (\Sigma t_{tb} - i)/430$	$0 \leqslant f_{lv} \leqslant 0.75$ leaves
$f_{tb} = (\Sigma t_{tb} - i)/430$	$0 \leqslant f_{tb} \leqslant 1.00$ tubers
$f_{st} = 1 - f_{lv} - f_{tb}$	stems
$f_{rt} = 1 - f_{shoot}$	fibrous roots
$i = 1/(0.0015 + 0.00079\,m)$	time of onset of tuber filling (°Cd)

Soil moisture balance of rootable zone

$\Delta S = Rain - E - T - Perc$ — daily change in soil moisture content ($mm\,d^{-1}$)

$T_0 = 1.1 \times ET_r \times (1 - \exp(-0.7k \times LAI))/(1 - \exp(-0.7\,k \times 4))$ — potential transpiration of the crop ($mm\,d^{-1}$)

$ET_r = 0.65 \times Rad \times s/(\lambda s + \lambda\gamma)$ — evapotranspiration ($mm\,d^{-1}$) of short grass as a function of solar radiation (Rad), slope (s) of saturated vapour pressure curve, psychrometric constant (γ), and latent heat of vaporization (λ)

$T = T_0 \times f_{WT}$ — actual transpiration ($mm\,d^{-1}$)

$E_0 = 0.94 \times ET_r \times \exp(-0.7k \times LAI)$ — potential soil evaporation ($mm\,d^{-1}$)

$E = E_0 \times f_{SM2}$ — actual evaporation as a function of soil moisture pressure in top 2 cm ($mm\,d^{-1}$)

Perc — moisture above field capacity drained away ($mm\,d^{-1}$)

Crop-water relations

$f_{WT} = (p_{wp} - p)/(p_{wp} - p_{cr})$ with $0 \leqslant f_{WT} \leqslant 1$ — transpiration reduction as a function of actual suction (p), critical suction (p_{cr}) and wilting point (p_{wp})

$p_{cr} = 400$ ($ET_0 = 5$) to 600 cm ($ET_0 = 1$ mm), $p_{wp} = 8000$ cm

$R_W = 0$ for $\Sigma t_{lv} < 725$ — relative leaf death rate (d^{-1}) due to water stress

$R_W = 0.03 \times (1 - f_{WT})$ for $\Sigma t_{lv} \geqslant 725$

$f_{WL} = 1 - 0.4 \times (1 - f_{WT})$ — reduction of SLA due to water stress

$(f_{tb} + f_{rt})/(f_{st} + f_{lv})$ = function of f_{WT} below-ground/above-ground partitioning

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 163–166.
PLSO PN 147
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Influence of genotype and water stress on the uptake of potassium and nitrogen in maize

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Key words: macroelement dificiencies, maize hybrids, nitrogen potassium uptake, water stress

Abstract

Three maize hybrids grown under condition of deficiencies of macroelements were subjected to moderate and severe water stress. Potassium and nitrogen uptake and release in the following 72 hours after onset of the stress show that at moderate dehydration, clearly expressed differences between the hybrids are observed due to their inherited particularities. At severe water stress, the significance of these differences decreases.

Introduction

Water deficiency exerts an influence on metabolic processes of plants and the changes in them are quite different, depending on the strength of the dehydrating factor. It has been established in maize that when moderate water stress is induced by polyethylene glycol, potassium and nitrogen uptake decrease in comparison with the control. At severe water stress intensive release of these elements in the zone of the roots is observed, but recovery of uptake is possible after the interruption of the unfavourable stress (Andonova, 1984; 1987). It is assumed that these results are due to the influence that the polyethylene glycol, as a dehydrating factor, exerts on the cell membranes of the root and consequently on capacity for membrane transport (Andonova, 1987; Cramer and Spurr, 1985). The purpose of the investigation was to establish how maize hybrids differ in their response to water stress and to the combination of water stress with nutrient deficiency.

Materials and methods

Three maize hybrids with different durations of their vegetative period (PH32 — with early maturity, BC6625 — mid-late maturity and H708 — late maturity) were grown for up to 1 month in solution culture on Helrigel's full nutrient solution with addition of trace elements from A to Z after Hoagland and chelating iron (FNS), and in solutions without nitrogen, sulfur, phosphorus, potassium, calcium and magnesium. The plants were then subjected to moderate and severe water stress for 24 hours, their roots being submerged in a solution of polyethylene glycol (M.W. 6000) with different concentrations (5 and 10 bars). In the following 72 hours the uptake of potassium and nitrogen, by those plants which were put into the full nutrient solution was measured by analysis of the nutrient medium.

Potassium was determined with a flame photometer, and nitrates by addition of Devarda's mixture and subsequent destillation (Andonova, 1987).

Results and discussion

a) Influence of water stress on potassium uptake by maize hybrids

All three hybrid comparisons of dehydrated and normal plants showed that water stress decreases the uptake of potassium (Fig. 1). The highest absorption of potassium is observed in PH32,

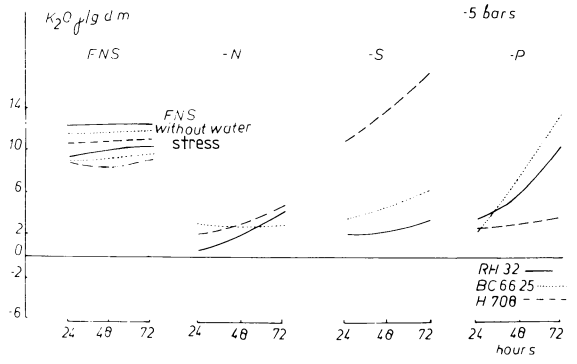

Fig. 1. Influence of FNS, N, S and P on K uptake of maize (moderate water stress).

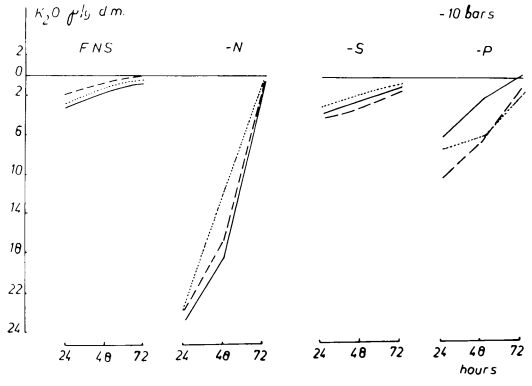

Fig. 2. Influence of FNS, N, S and P on K uptake of maize (severe water stress).

somewhat lower in BC6625 and the lowest in H708. After moderate water stress, the day-and-night course of potassium uptake hardly changes its characteristics and at the end of the experimental period a small increase in uptake is observed. At moderate water stress the uptake changes are considerable when it is combined with a preliminary nutrient deficiency.

The influence of anion deficiency on potassium uptake after a moderate dehydration is different in all three experimented hybrids. In general, a different reaction is observed at the beginning and the end of the experimental period. After 24 h of the dehydration and nitrogen deficiency, potassium uptake is more reduced in PH32 and H708 than in BC6625. However, at the end of 72 hours the intensity of potassium uptake in the last hybrid remains almost the same, while in the first two hybrids it increases significantly. Specific differences occur when phosphorus deficiency is taken into consideration, the H708 hybrid being the most sensitive. When sulfur is deficient in the same hybrid, stimulation of potassium uptake is observed, greater than the control plants grown on full nutritive solution.

All these plants and nutritive variants under conditions of severe stress have shown release of potassium in the zone of the roots and this release progressively decreases with a tendency to restoration of uptake after about 72 hours (Fig. 2). In most variants of deficiency, the reaction of the experimented hybrids is slightly different, but the influence of the preliminary availability of nutritive elements is greater. The highest release of potassium after dehydration occurs in the nitrogen deficient solution. When sulfur is deficient, potassium release is low, as in plants grown in complete nutrient solution. Potassium uptake after about the 72nd hour is not yet restored, and only approaches the point of compensation. When phosphorus is deficient at the beginning, the most sensitive hybrid is again H708, as shown at moderate water deficit. During the later period, in the variant with phosphorus deficiency, the differences between the hybrids decrease. The results given in Figure 2 have the following common characteristic: however great the difference between the variants may be (and sometimes these differences are over double) about the 72nd hour potassium release decreases and approaches the point of compensation. That

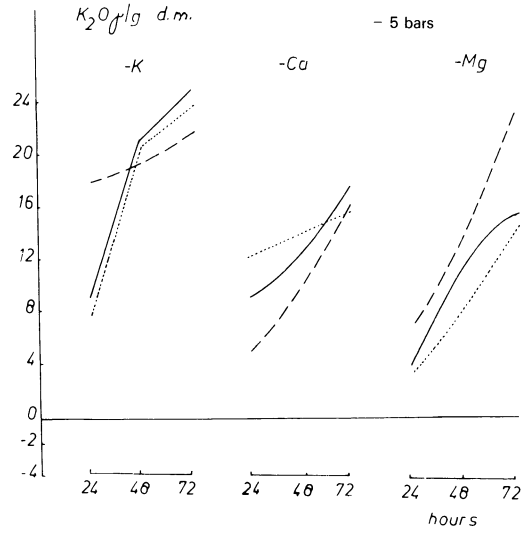

Fig. 3. Influence of K, Ca and Mg on K uptake of maize (moderate water stress).

indicates that the regulation of the recovery processes related to potassium uptake is slightly influenced by hybrid differences and exogenous actions and is probably dependent on the species. Moderate water stress combined with cation deficiency exert an unequal influence on potassium uptake in the hybrids (Fig. 3). The hybrids with earlier and middle late maturity under potassium and magnesium deficiency show a significant decrease of potassium uptake at the beginning. This uptake is greatly stimulated about the 72nd hour under the combined conditions of potassium deficiency and moderate dehydration. The hybrid H708 is less sensitive to potassium and magnesium deficiencies but possesses the greatest sensitivity under calcium deficiency. Regardless of these various reactions of the hybrids with respect to potassium uptake, atbout the 72nd hour in all three hybrids a stimulation of potassium uptake is observed and this uptake is superior to that of the dehydrated and normal controls. This phenomenon can be explained by the interaction between the cations, when the deficient cation is intensively absorbed when restored to the nutrient media, on the one hand, or, on the other hand, it can be substituted to some extent by some other cation. In our case, all respective elements have been returned and after calcium and magnesium deficit, we probably have to do with synergic effect on potassium uptake (Abutalybov *et al.*, 1984; Palladina, 1984).

At severe water stress and conditions of preliminary deficit of cations, release of potassium is observed in all three hybrids and in all variants, as in anion deficit (Fig. 4). About the 72nd hour an approximate or complete point of compensation is reached. It has been observed that under potassium deficits all hybrids go beyond the limit of this point but the differences between the hybrids are not clear. The slowest restoration of plants has been observed during magnesium deficiency; it is quicker at calcium deficit and it is most quick at potassium deficit.

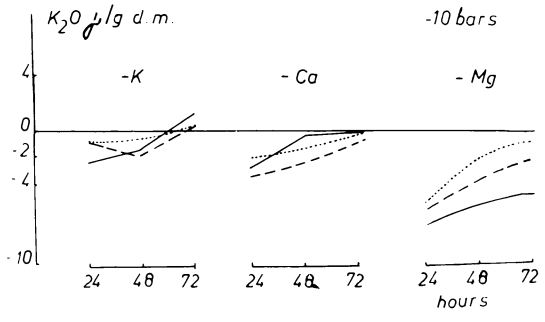

Fig. 4. Influence of K, Ca and Mg on K uptake of maize (severe water stress).

b) Influence of water stress on nitrogen absorption by maize plants

Figure 5 shows nitrogen uptake at the 72nd hour after moderate and severe water stress. Uptake of nitrogen like potassium in dehydrated plants (FNS) is reduced in comparison with the control plants, this reduction being very clearly expressed at severe stress. After severe water stress nitrogen uptake in almost all variants is restored. That is an evidence that nitrogen uptake, in comparison with potassium uptake, is relatively more resistant to water stress. After a moderate water stress and nutrient deficit stimulation often occurs, this effect being evident in all variants for H708. However, nitrogen uptake in PH32 is severely hampered when nitrogen and potassium are deficient. An intermediate position occupies BC6625 which at moderate dehydration and nutrient deficit shows a resistance to nitrogen uptake. That is, to some extent, confirmed at severe water stress for the same hybrid, nevertheless about the 72nd hour this hybrid in all deficiencies has overpassed the point of compensation of nitrogen uptake. This point, in potassium and magnesium variants, is not reached by the two other hybrids and for the hybrid PH32

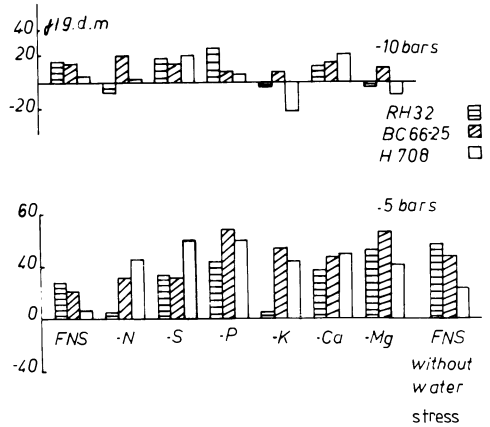

Fig. 5. N uptake of maize at nutrient and water deficiency.

this point is not reached in nitrogen deficiency either. These results show a clearly expressed difference in the nitrogen metabolism of these three hybrids probably due to the regulation of the enzyme systems (Cacco *et al.*, 1982).

The capacity for nitrogen absorption is more resistant than potassium under similar conditions of nutrient and water deficiencies. The hybrids with mid–late maturity show a greater resistance of nitrogen uptake at nutrient and water deficiencies than the hybrids with early and late maturity. In H708 moderate dehydration combined with nutrient deficiency stimulates nitrogen uptake, a phenomenon which can be observed at the 72nd hour of the recovery period. As with the potassium variant at severe water stress the differences between hybrids are more slightly expressed than in moderate water stress.

Plant resistance to drought is connected with the resistance of the individual cell structures to dehydration (Wolfe, 1987). It is well known that the reversibility of processes after unfavourable conditions depends on the maintenance of their integrity and functional activity (Michalik, 1974; 1984). The results obtained in our investigation show that beside the exogenous factors, the so called threshold of resistance is connected with the inherited characteristics of maize hybrids and within the limits of moderate water deficiencies they are the origin of the observed differences in potassium and nitrogen uptake by hybrids with different duration of the vegetative period.

Conclusion

Under moderate water stress, potassium and nitrogen uptake is reduced in comparison with the normal plants. The degree of that reduction depends on the preliminary availability of inorganic nutrients. The differences between the reactions of the hybrids are clearly expressed when they are subjected to a combination of nutrient and water deficit. The uptake of investigated elements is restored at about 72 hours.

Under severe water stress, 24 hours after the treatment, in the zone around the roots, instead of absorption, a release of potassium and nitrogen is observed. That release progressively diminishes and around the 72nd hour turns, in some variants, to reabsorption. The genetic differences between the hybrids are expressed very slightly. Potassium uptake being more strongly influenced by water stress and nutrient deficiency than that of nitrogen.

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PLSO PN 148

Salinity tolerance of different halophyte types

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Key words: halophytes, salinity, tolerance

Abstract

The salinity tolerance of plants exhibits a great importance in agriculture of arid areas where the salinization of soils is enhanced by irrigation in an increasing scale. Arid areas in this respect are problem areas. In humid regions the salt-balance in natural ecosystems as well as in agro-ecosystems is much more favourable. Cultivated plants, used by mankind as crop-plants, are almost all rather salt-sensitive. Plants growing naturally on saline stands have evolved various mechanisms to cope with salinity.

Several types of halophytes can be distinguished. Chemically defined halophyte types are not so clearly arranged on a salinity tolerance scale than ecophysiologically and/or anatomically-morphologically defined plant types. Thus, especially halo-succulent and salt-recreting plants are the main groups, other than the highly selective grasses. Within these groups, the concept of ecotypes has been used. It is obvious, that taxonomic relations of the halophytes give indications for a typical genetic background of salinity tolerance which, however, is very complex. This leads to altered and more precise descriptions of the phenomenon 'salinity tolerance'. The latter has to be regarded in more than one aspect on the levels of the organell, the cell, the plant tissue, the organ and the whole individual plant. An outlook to agricultural perspectives is given.

Introduction

The salinity tolerance of plants exhibits a great importance in agriculture of arid areas where the salinization of soils is enhanced in an increasing scale by irrigation. Arid areas in this respect are problem areas. In humid regions the salt-balance, in agro-ecosystems as well as in natural habitats is much more favourable.

Cultivated plants, used by mankind as crop-plants, are almost all rather salt-sensitive. On natural stands, especially along the sea-coast in all continents, as well as in inland areas in arid regions a variety of halophytes occur. In inland areas of humid regions saline stands occur only very locally, for instance around salt-springs.

We have to ask first: what is salt-tolerance? Second: what are the mechanisms of salt-tolerance and what is the behaviour of halophytes?

Only when these questions are more clarified will there be a chance to consider possibilities of improving crop plants in their salinity tolerance or to establish new salt-tolerant crops.

Halophyte-types

Various types of halophytes have been described in the past and various definitions used to separate them from each other. Some typification of a variety of parameters have been used and it is very essential to define those parameters (Waisel, 1972). A survey on classifications used is given in Table 1.

The various approaches indicate that the types to be defined as halophyte-types may vary and sometimes plant species can be grouped under more than one category. Furthermore, the point of view may vary and it is of importance which hierarchic level on the organismic scale is applied.

In the way used here, as to discover the corre-

Table 1. Classification of halophytes. Sequence: author, used parameters, special types distinguished and terms coined

Stocker (1928): water supply; Xero-, Meso-, Hygro-Halophytes
Chapman (1942): soil salinity; Mio-, Meso-, Mesoeu-, Eueu-Halophytes
Eijk (1939): response to salinity and habitat; tolerating; demanding salt—in nonsaline; saline habitata
Tsopa (1939): response to salinity; obligatory; preferential, supporting; accidental
Walter (1968): dominant anions in cellsap; Chloride-; Sulphate-; Alkali-
Duvigneaud and Denayer-De-Smet (1968): dominant elements in ash; Halophore; Halothiophore; Potassionitrophore and others
Harmer *et al.* (1953): response to Na under K-deficiency or K-supply
Steiner (1935): internal salt balance and morphology; succulent; nonsucculent; accumulating
Henkel and Shakov (1945), Soloviev (1967): salt and osmotic regulation; Euhalophytes; Crino-; Glyko-; Non-
Breckle (1976): salt balance; Eu-(stem-, leaf-succulents); Crino-(endo-, Exo-); Pseudo-; Non-
Albert and Kinzel (1973): physiotypes; gramineous; chenopodiaceous; *Lepidium*-; *Plantago*-
Waisel (1972): narrow or wide range of salinity; stenohaline; euryhaline
Waisel (1972):plant–salt relationships and internal salt; Eu-; Pseudo-Halophytes; salt-requiring; salt-resistant; obligatory; preferential; salt-enduring; salt-excluding; salt-evading; salt-avoiding

lations of distinct halophyte types and their salt-tolerance it is most promising to use an ecophysiological approach in defining types of halophytes and to try to give hints on the adaptations and strategies of those plants.

In Figure 1 we give a schematic diagramm of processes which may be more or less responsible and decisive for the relevant halophyte type. Five main types of halophytes are distinguished which again in Table 2 are shown. The most effective ones are those, which apply the more specific and more effective control mechanisms for maintaining constant ion levels of Na and/or Cl, or other excessive ions.

The lower part of Table 2 gives examples of genera which exhibit the regarded mechanisms most obviously. Most of the halophytic genera can be placed distinctly in one of these groups. Crop-plants, however, are not listed here, they belong almost exclusively to the non-halophytes, only few examples are known to be real pseudo-halophytes, as *e.g.* members of the genus Beta, Spinacia, Brassica *etc.*

Salt tolerance and salt resistance

It is generally accepted (Levitt, 1980) that the tolerance against a specific ecological factor such as drought, frost, heat, salinity is the more specific term for describing a distinct adaptation of a plant species in coping with the relevant stress-factor, whereas resistance is the overall general term. Levitt (1980), suggests eight stresses which gave rise to resistance adaptations of plants during the long times of evolution (see Fig. 2). Table 2 indicates three main categories by which plants may reach a real or seeming higher salt resistance: avoidance, evasion (which is sometimes included in avoidance) and tolerance.

The term salt tolerance is sometimes used in literature without determining whether or not the stress is tolerated internally in the plant. However, there are uncertain cases, indicating that either avoidance or tolerance is involved. All such uncertain cases should be called 'resistance'. Salt resistance can be defined, as is shown in Table 3 in various ways.

Of specific importance is the observation of ecotypic variation with coastal and inland ecotypes. Waisel (1972) has given several examples from the flora in Israel and in Central Europe several examples are known, too. There exist in many species ecotypes which are adapted to higher salinities. Inland and coastal ecotypes are known from many species. They differ often in flowering time and in their growth parameters.

In a comparative survey Breckle (1986) has given a list of families in the Flora Europaea Region on one side and the Flora Iranica Region on the other

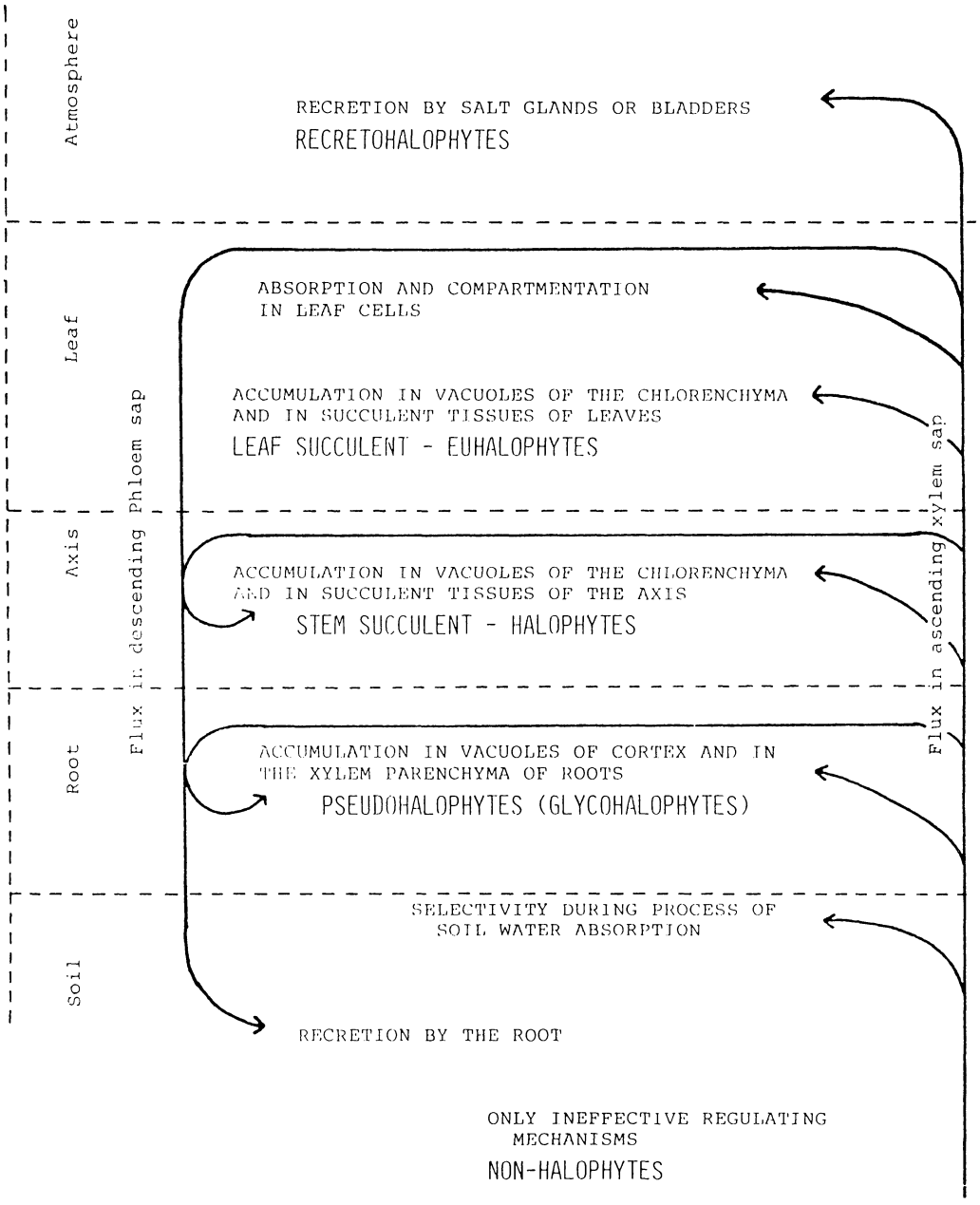

Fig. 1. Halophyte types characterised by the dominance of processes regulating salt concentrations in plant organs and accumulation and recretion sites of salt. Scheme of water and NaCl uptake. (Breckle, 1976).

side, indicating the total number of genera and species and the halophytic ones. It revealed that mainly the Chenopodiaceae contain the largest number of halophytes in both regions, on the genus and species level. Ranking second are the Poaceae, but only in absolute figures, not on the percentage level. This is true also for the Compositae ranking third.

A more general viewpoint should be stressed, *e.g.* in the subclass Caryophyllidae there are some plant families which exhibit special adaptations for mineral metabolism. Heavy metal tolerance in

Table 2. Control mechanisms in plants for maintaining a constant level of sodium ion concentration in cell-sap of plant tissues (particularly in chlorenchyma cells) and main types of halophytes

A) Mechanisms	
1 Avoidance	
a Growth only during favourable seasons	
b Growth only on favourable sites	
c Limitation of root growth and root absorption activity to distinct soil horizons	
2 Evasion	
a Selectivity against Na and Cl	
b Leaching of salt out of the shoots	
c Diversion of salt out of the assimilating tissues	
d Compartmentation of salt within plant, tissues, cells	
e Re-translocation of salt to roots and recretion by roots (?)	
f Disposal of older plant parts ('salt-filled organs')	
g Recretion by gland-like structures on shoots	
i salt-glands	
ii bladders	
3 Tolerance	
a Increasing of salt resistance of tissues, cells, organelles	
b Increase in halo-succulence	
i increase in leaf-succulence	
ii increase in stem-succulence, reduction of leaves	
B) Types of Halophytes (and examples of genera in italics)	
mechanisms more or less in all plants: especially important in Non-Halophytes (N)	1, 2a–d;
mechanisms most characteristic in Pseudo-Halophytes (P) (*Juncus*, rosette-plants)	2d–f; partly 3a
mechanisms most characteristic in Recreto-Halophytes (X)	2g
in Exo-Recreto-Halophytes: (*Cressa*, *Frankenia*, *Limonium*, *Tamarix etc.*)	2gi
in Endo-Recreto-Halophytes: (*Atriplex*, *Halimione*, *Mesembryanthemum* s.l. *etc.*)	2gii
mechanisms most characteristic in Euhalophytes:	2d–f, 3a, b
in Leaf Succulent Euhalophytes (L): (*Salsola*, *Suaeda*, *etc.*)	3bi
in Stem Succulent Euhalophytes (S): (*Salicornia*, *Halocnemum*, *Halostachys*, *etc.*)	3bii

Plumbaginaceae and in Caryophyllaceae, as well as salinity tolerance in the same families is obvious, however, in other species and genera.

Salt responses of halophytes

As was already shown in Table 2, halophytes exhibit various mechanisms in coping with salinity. A few more detailed remarks and additional examples will be given in the following.

Hints of gradual salinity tolerances are available if salt gradients along borderlines of natural salt pans in deserts or along saltlakes are examined. It turns out, that in areas with a rich halophytic flora as *e.g.* in Iran, Afghanistan, Central Asia, Great Basin in USA, many plant communities develop along these gradients. Their richness in species is very variable, but indicative. It can be concluded from such gradient studies, when compared with thorough analysis of soil parameters, that there is, as a general rule, a distinct zonation of halophyte types, which was termed the S-L-X-P.N sequence (Breckle, 1986). This means, that, (as in Figure 3 in an example from the USA and in Table 4 from the Middle East shown) along the salt gradient, it is determined by the different salinity resistances of the species and by the overall species competition how the sequence is filled with halophytic communities under long term equilibrium conditions.

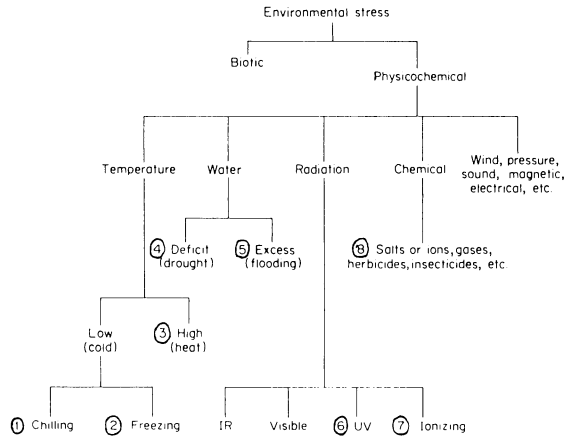

Fig. 2. The eight stress types as defined by Levitt (1980) to which organisms may be subjected. Resistance mechanisms against each of the numbered stresses have been identified.

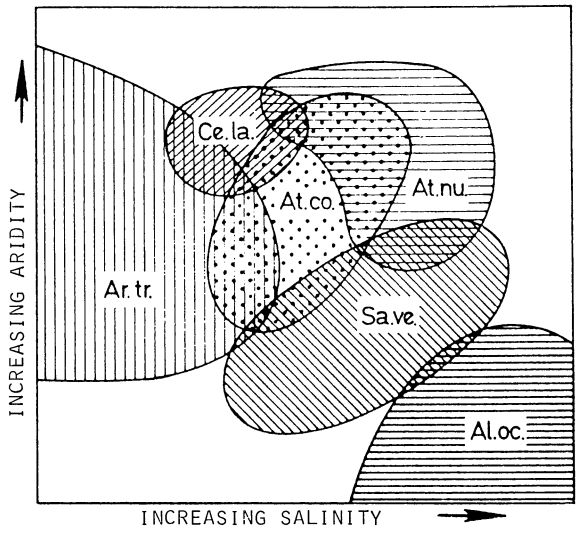

Fig. 3. The sequence of dominant halophytic species along the salt gradient near Great Salt Lake, Utah, shown in an ecogram with salinity and aridity (Breckle, 1976). The gradient goes from lower right to upper left. Al. oc = *Allenrolfea occidentalis*; Ar. tr. = *Artemisia tridentata*; At. co. = *Atriplex confertifolia*; Ce. la. = *Ceratoides lanata*; Sa. ve. = *Sarcobatus vermiculatus*.

Table 3. Salt resistance, acc. to Hayward and Wadley (1949), Richards (1964), Waisel (1972), Breckle (1976) and Albert (1982)

1. the ability of plants to survive and to complete their life cycle under saline conditions.

   Ecological aspect
   survival ability, endurance, criterion for wild species; growth, size, yield is neglected

2. the relative ability of a species to grow on saline soils in comparison to its growth on nonsaline, optimal conditions.

   Physiological/Ecophysiological aspect
   determines adaptability of species more than their ultimate resistance

3. the relative ability to grow and yield on saline soils in comparison to other species.

   Agricultural aspect
   determines yield e.g. or 50% reduction, but not competitive abilities *e.g.* under natural conditions

In any way salt resistance has at least 2 cardinal points. The one is the upper limit of either growth of a plant or existence of a plant species.

The problem is, that salt resistance varies very much. It varies:

1 during different growth or development phases of a plant.
2 with ionic pattern in soil
3 with life form and halophyte type
4 with the plant organ affected by salt
5 within one species forming ecotypic varieties

The succulent halophytes are either leafless and stem-succulent, or the leaves are very fleshy and succulent. These halophytes normally exhibit the highest salinity tolerance. But there are the so-called xero-succulents, as *e.g.* cacti, Euphorbia, Sedum, Sempervivum *etc.* which should be strictly distinguished from the halo-succulents, mentioned before. In a few cases, there are however intermediates in a few genera known, as *e.g.* in the Mesembryanthemaceae or Aizoaceae, also a very few *Opuntia* species seem to be somewhat salt-tolerant, too. In the typical halophytic stem-succulents, as Salicornia, Arthrocnemum, Halocnemum *etc.* it is thought, that the high salt-concentrations are mainly restricted to the large vacuoles in the storage tissue cells. If this is a real mechanism of diluting the salt-concentration sufficiently seems rather unlikely.

The recretion of salts was very intensively studied in many examples. The salt-glands on one hand, and the saltbladders on the other hand, are structures, which are more or less able to reduce the salt in the plant body by an appreciable amount. The salt-glands in Limonium start to be active only, if there is some salt stress. As shown in Figure 4 Na and Cl concentrations in the leaves of *Limonium*

Table 4. Percentage values of calculated abundancies of halophytic plant types arranged according to salinity levels (logarithmic intervals), averages from 8 halophytic zonations in Iran and Afghanistan (S, L, X, P and N, see Table 2B)

	Halophytic plants (%)											
NaCl (%)	15 – 10	– 5	– 3	– 2	– 1	– 0.5	– 0.2	– 0.1	– 0.05	– 0.02	– 0.01	– < 0.01
S	71	63	51	18	3	12	7	4	11	10	0	0
L	29	32	30	72	30	56	51	18	35	18	10	0
X	0	5	19	0	60	27	28	41	4	9	0	2
P	0	0	0	5	7	5	15	23	24	45	68	10
N	0	0	0	0	0	0	0	14	26	24	22	88

sinuatum have increased with increased salt in nutrient solution. But the material eliminated by the salt gland only starts to contain Na and Cl in appreciable amounts, when concentration of the nutrient solution contains more than 30 mmol of salt.

The ion specific mechanism of recretion is different from species to species. *Limonium sinuatum* is not as selective for potassium and sodium as *L. gmelinii* and *L. ramossissimum*, cultivated in nutrient solution of various K/Na ratios (see Fig. 5, where the ratio is given as K/(K + Na)). The relevant ratio indicates, that in leaves K is favoured, in the recreted fluid Na is dominant; the leaves are, thus, kept low in Na, but high in K-concentration.

Another example is shown with Chenopodium, a very widespread genus, but not typically halophytic. Most species have a typical indumentum with bladder like hairs, which resemble those from the genus Atriplex. From that genus it is known that these bladders are able to store high concentrations of NaCl in the vacuole (Osmond, 1980; Mozafar and Goodin, 1970). In Chenopodium this similar structure of bladders is also able to recrete salts on a lower scale. This was shown by Reimann and Breckle (1987a; b): the halophytic character is still recognizable, even in plants, growing in non-

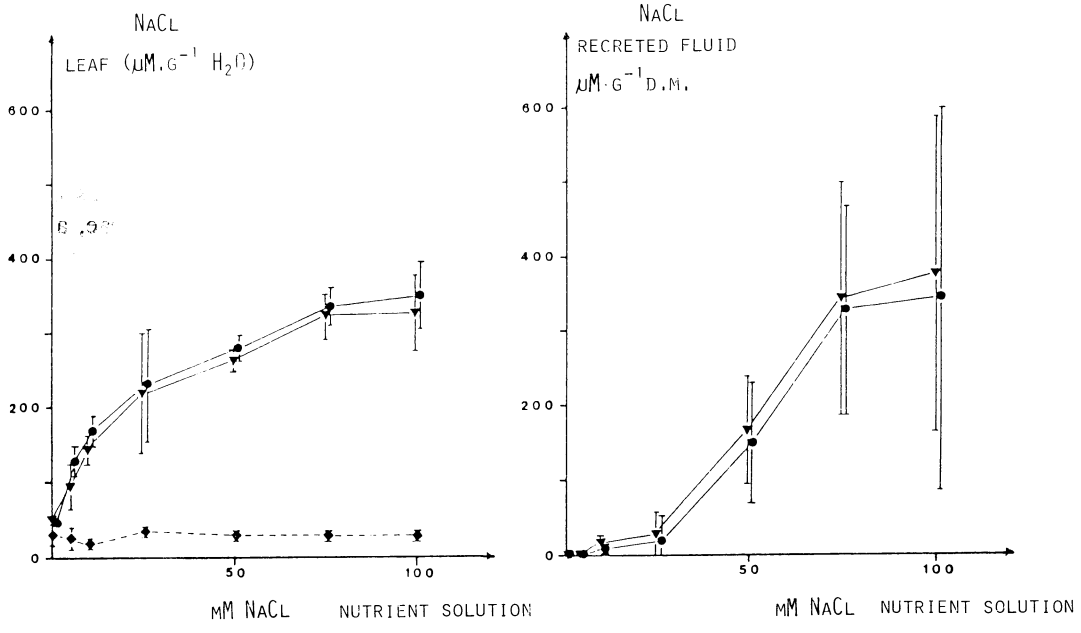

Fig. 4. Left: Na-, K-, Cl- concentration in leaves of *Limonium sinuatum* (possessing salt-glands) with increasing salt in nutrient solution. Right: Na- and Cl- concentration in recreted fluid from salt glands on the leaves in *Limonium sinuatum* with increasing salt in nutrient solution (Wiehe, 1986). ●: chloride; ◆: potassium (K^+); ▼: sodium (Na^+).

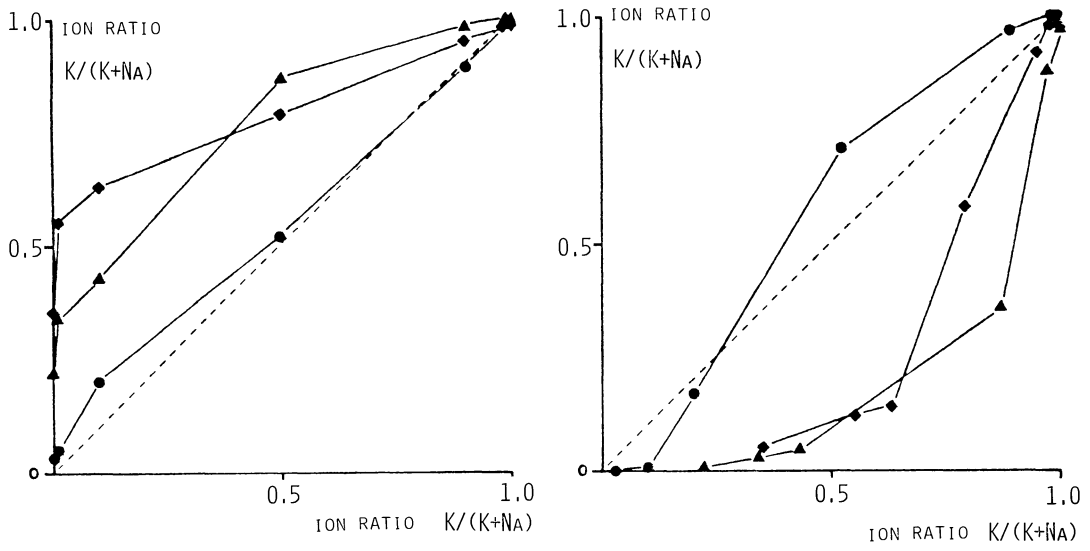

Fig. 5. The ionic ratio ⟨K/(K + Na)⟩ in leaves (left) and in recreted fluid (right) in *Limonium sinuatum* (●), *L. ramossissimum* (▼) and *L. gmelinii* (◆) with changing ratio in the nutrient solution (Wiehe, 1986).

saline habitats in nature. As an example the response of three species of *Chenopodium* to salinity is shown in Figure 6. Regardless which parameter is used it is obvious, that growth of *C. giganteum* and *C. murale* is enhanced by NaCl, where *C. album* decreases growth when cultivated with increasing salt concentrations in nutrient solution.

At the level of the cell the salinity response was tested by Repp *et al.* (1959), (Repp, 1964) with sections of leaves or stems and the Plasmolysis reaction recorded to evaluate some kind of plasmic resistance against salt. It turned out that cells are able to adapt rather quickly to salt stress.

In tissue cultures and suspension cultures meristem cell lines could be cultivated under increased salinity stress. It was shown by von Hedenström and Breckle (1974) that the response curves of the highly salt tolerant Salicornia or Suaeda, were

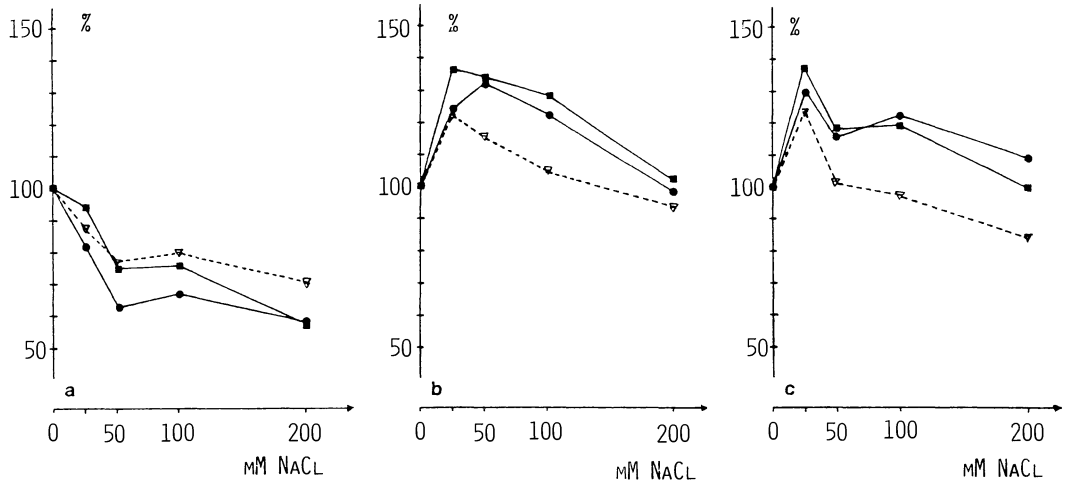

Fig. 6. Growth parameters (relative growth rate as dry matter ⟨—●—⟩, fresh weight ⟨—■—⟩ and shoot length ⟨--▽--⟩, % of control plants in saltfree medium) of *Chenopodium album* (**a**), *Ch. giganteum* (**b**) and *Ch. murale* (**c**) with increasing NaCl in nutrient solution (Reimann and Breckle, 1986a; b).

Table 5. Effects of NaCl

1. Biochemical effects: enzyme activities, halo-enzymes
2. Effect on membranes: membrane-bound enzymes, electric potentials, active transport
3. Effects on cell organelles: respiration, photosynthesis, salt-resistance, secondary compounds
4. Interaction with cells and tissues: formative effects, succulence, compartmentation, salt-resistance
5. Interactions with individual plants: ecological adaptations, salt-tolerance, salt-resistance
6. Interactions in and with ecosystems: cycling of salt, zonation along salt-gradient, competition; budget: pools and fluxes
7. Interactions in biomes, biosphere: transmission, cycling, accumulation; budget: pools and fluxes

The levels 1 and 2 are subjects of biochemistry; 2 and 3 of plant physiology, partly 3, mainly 4 and 5 of ecophysiology, 5 and 6 of ecology, 6 and 7 of ecosystems research and global ecology.

rather similar to those of the salt sensitive Phaseolus. It was concluded that salinity tolerance of a whole plant is a complex phenomenon, not only related to the tolerance of cells. Similar conclusions were drawn by R. Munns/Canberra (pers. communic.) from relevant experiments with other species.

Salinity tolerance apparently has to be defined as a property of a whole intact plant, where all organs function in harmony together. A whole system of physiological characters and reactions is involved.

Conclusions

The effects of salt are on various levels. In Table 5 there are given examples from the biochemical level (membranes *etc.*) up to the almost global level (climate and atmospheric cycling).

To improve the situation in arid areas with respect to salinity in agriculture it is obvious that the solution is not simple and must be examined on various levels. Leaching of salts from soil, improving ion balance, improving water balance, but also improved salt tolerance of distinct ecotypes of crop plants with least affected photosynthesis *etc.* should be applied.

For agricultural purposes it is hoped to find salt-resistant plants as good crop plants with high yields. In natural halophytes with relatively high yield there might be candidates in the general Atriplex and Chenopodium in the Chenopodiaceae, Amaranthus in Amaranthaceae or Inula in Asteraceae mentioned, but also in Mesembryanthemaceae and Aizoaceae there might be promising genera.

The grasses play a special role having their own adaptations (confer the heavy metal tolerance, Fe-uptake by siderophores *etc.*), as well as in salinity adaptations. They apply very selective mechanisms in their roots for uptake of minerals.

The energy costs for compartmentation at the cell-level or for salt-recretion or for other strategies to cope with salt are certainly in the same range with only slight differences. Not only one way is thus promising in improving crop plants in their salinity response. Even contradictory mechanisms may be applied by natural halophyte types in a combination of strategies. We are still not far from the starting point of a long way to understand how halophytes cope with salinity and how this could be used to improve crop plants in their salinity tolerance.

As there are many processes involved in polygenic patterns, there is also no straight way for plant breeders. Salt resistance is a quantitative characteristic of plants which is determined by a large number of genes. Salt resistance depends upon a variety of plant traits and involves the mechanisms of uptake, transport and elimination of ions. It also depends upon the various responses of the metabolism to specific ions (Levitt, 1980;

Waisel, 1972). Apparently all the aspects of the hydration of the plant, including water content, rate of transpiration and degree of hydration of cytoplasm or of specific chemical compounds also contribute to the determination of salt resistance (Malkin and Waisel, 1986; Oertli, 1976). Genetic research may reveal many new ideas on the basics of halophytism and may throw more light in the complexity of the various halophyte types known at present.

Acknowledgements

Studies were supported by a joint research programme of the Universities of Bonn and Kabul (Iran, Afghanistan), by Schimper Fellowships for extra-European ecological studies (Tunisie, Iran), by DFG-grants (Australia, USA) and by research funds of the University of Bielefeld (Israel, Spain, South Western parts of Africa). This is very much appreciated. For the kind hospitality of the local people and for their help we are very grateful.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 177–182.
PLSO PN149
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Physiological differences between barley cultivars under salt stress – xylem exudation and phloem flow of different cations

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Key words: barley, cation, phloem, salt-stress, transport, xylem

Abstract

Different barley cultivars were exposed to a salt-stress of 100 m*M* NaCl, in hydroponic conditions, one week after germination using diluted Hoagland solution for nutrition. Furthermore the effect of the presence or absence of calcium was analyzed, using a calcium-less or a calcium-containing solution. Ion uptake was followed for 48 hours, growth as an increase of dry matter was measured as was the ionic content for potassium and sodium. In parallel with this uptake experiments we followed the sodium fluxes, using ^{22}Na, to determine ratios of accumulation of xylem transport. All cultivars were also separately exposed to the stress for half an hour, after which the phloem transport of sugars was measured. Having data on several physiological parameters we have been able to classify the different cultivars from relative resistent to sensitive to salt-stress.

Introduction

From earlier works on salt-stress (Bogemans and Stassart, 1984; Stassart and Bogemans, 1984) we had found some significant differences relative to the resistence of several barley cultivars to salt-stress, but these findings were based on results obtained after long term experiments, a full growing season (Bogemans and Stassart, 1987; Stassart and Bogemans, 1987). Our aim was to find out if salt-sensitivity could be detected much earlier in the life of the plant. The detection of failures due to stress on a 24 to 48 hour basis needed more physiological data, therefore it was necessary to follow ^{22}Na through roots (Neirinckx and Stassart, 1979) to detect eventual collapse of the ion transport selectivity (Stassart *et al.*, 1987; Stassart, in press). As a plant is exposed to a salt or osmotic stress it will have to react by accumulating solutes. It was found that barley accumulated proline in the long run (Bogemans and Stassart, 1983), but at short notice it seemed evident that a glycophyte would use sugar as a readily available solute which can be transported easily over the whole plant (Boschmans and Stassart, 1985). The fact that a plant would use sugar to compensate for an applied salt-stress, means it uses a considerable amount of energy to fight the unfavourable conditions. By doing so, the reallocation of sugar, it will suffer at another level, meaning it should grow more slowly. Thus growth reduction would be a secondary effect of salt-stress.

Material and methods

Barley seeds were germinated in moistened sand overnight and placed on gauze over a 0.25 m*M* $CaSO_4$ filled tank to develop roots for 48 hours in the dark. Two days after germination, young seedlings were put into light (16 hours light cycles). One week after germination the $CaSO_4$ was removed at the end of the culture phase and replaced by 100 m*M* NaCl and 10 m*M* KCl or by 100 m*M* NaCl, 10 m*M* KCl and 5 m*M* $CaCl_2$. Good aeration was provided for this hydroponic treatment. Ion content of

Table 1. Cultivators used in the experiment

Number	Cultivar	Seed row	Type
1	Sonja	2	Winter
2	Candida	2	Winter
3	Regent	2	Summer
4	Fridpone	2	Summer
5	Auriga	2	Winter
6	Isaura	2	Winter
7	Union	2	Summer
8	Sigma	2	Summer
9	Igri	2	Winter
10	Atem	2	Summer
11	Iban	2	Summer
12	Menuet	2	Summer

roots and shoots was measured after 24 hours, from low-salt-plants to high-salt-plants, and after 48 hours. In parallel with this uptake experiment, young plants were taken out of $CaSO_4$ to a ^{22}Na labelled solution in transport-boxes. Further a batch of each barley cultivar was exposed to the salt solution, after half an hour roots were cut off and the cut-end of the shoots brought in 1 mL EDTA to collect phloem flow. This was analyzed for reducable sugars content.

The barley cultivars tested during the described experiments are shown in Table 1.

Analytical procedures

Cation content measured after dry-ashing in a muffle furnace at 560°C, dissolving ashes in 1.5 *M* NHO_3 and brought to volume by bideionized water, over a flame-photometer.

Radioactive tracer was measured in root and solution samples, after drying in planchettes, with a GM counter. Reducing sugars were measured in 1 mL 10 m*M* EDTA samples by the Anthrone method.

Results

Growth

Though growth is rather small over 48 hours, it can nevertheless indicate some tendency as how strongly a plant is affected by the applied salt-stress. The growth of one-week-old barley plants is quite vigourous so even small changes can be observed. Figure 1 shows results of growth as an increase in dry matter in roots and shoots for each cultivar after 48 hours for both salt treatments (plus or minus calcium). Values of dry weight at the end of the culture phase were set at 100% for each roots and shoots.

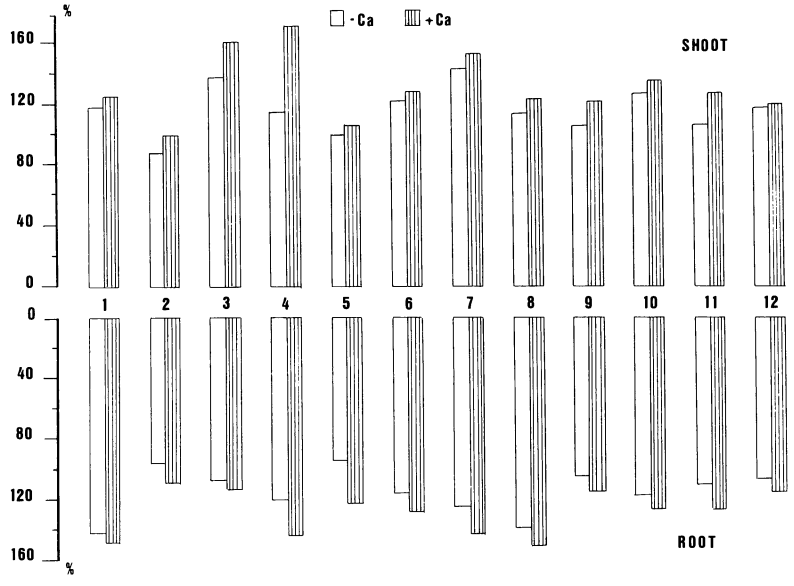

Fig. 1. Growth as a percentage increase of dry matter over 48 hours, for roots and shoots of salt stressed barley cultivars, depending on the calcium status in the outer medium.

From the data shown in Figure 1, one can draw the following preliminary conclusions: 1. The absence of calcium during the uptake reduces growth by about 10%, roots and shoots alike. 2. The decrease of growth due to the saline treatment is more pronounced in winter barley in general, this reduction was less obvious in the summer barley cultivars.

Ion content

Since cultivars had slightly different ion contents at the beginning of the experiment, we concentrated our findings on the net uptake of cations, mainly sodium and potassium. As the calcium content did not vary significantly during the 48 hour uptake period, data were not shown in the figures presented. Figure 2 shows data for the calcium-less treatment, it is clear that especially for winter barley the ion selectivity was near breakdown, allowing massive amounts of sodium to the shoot. Some summer barley cultivars could still manage to keep a higher potassium level in the shoots, compared with sodium. Figure 3 shows the data for the calcium-containing treatment. Here it is clear that the summer barley shows a better ion selectivity over the range of its cultivars. For the winter barley results show some differences, some performed well, but other cultivars were not able to refrain sodium to be transported massively to the shoot.

Xylem exudation

Using transport-boxes one can measure the accumulation in the root, the redistribution of the ion to the basal root end and its xylem exudation. Figure 4 shows results obtained for the different cultivars in both saline treatments, with calcium absent or present. On average winter barley cultivars absorb a larger quantity of sodium thus reaching a higher accumulation level and xylem-transport than found in summer barley. When repeating the experiment using calcium in the outer medium one observes a greater effect of calcium on the sodium fluxes in the sensitive winter barley than is the case in the summer barley. Though even then the sodium transport through the xylem keeps higher in the winter barley.

Phloem sugar transport

When exposed to a salt-stress barley will re-

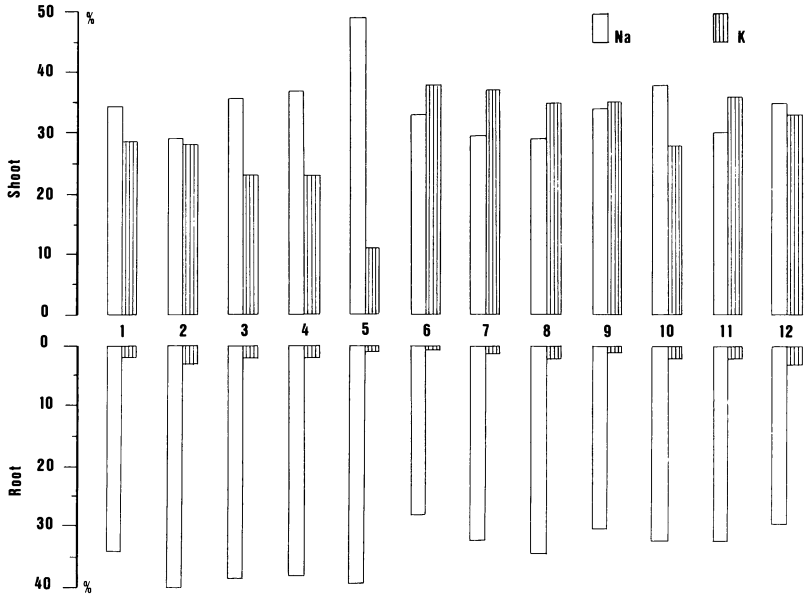

Fig. 2. Potassium and sodium accumulating in roots and shoots. The sum of which is set to be 100%, thus giving the apparent distribution of the cations in both plant parts. (calcium-less treatment)

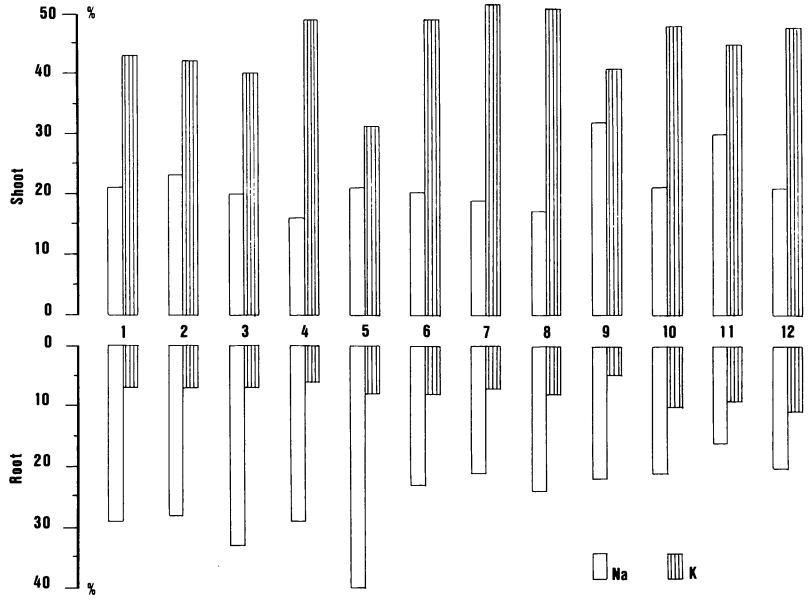

Fig. 3. Potassium and sodium accumulation and percentual distribution in roots and shoots of salt stressed barley cultivars. (calcium containing treatment)

lease more sugar from the shoot towards the roots, and that even after only a short contact of the roots with the NaCl solution. Figure 5 gives data on the measured sugar fluxes under stress situation, but also the normal sugar fluxes measured when plants were not subjected to a stress. From the data presented in Figure 5 it is clear that most winter barley cultivars show a much higher rate of sugar transport to their roots, but also a much larger increase as compared with

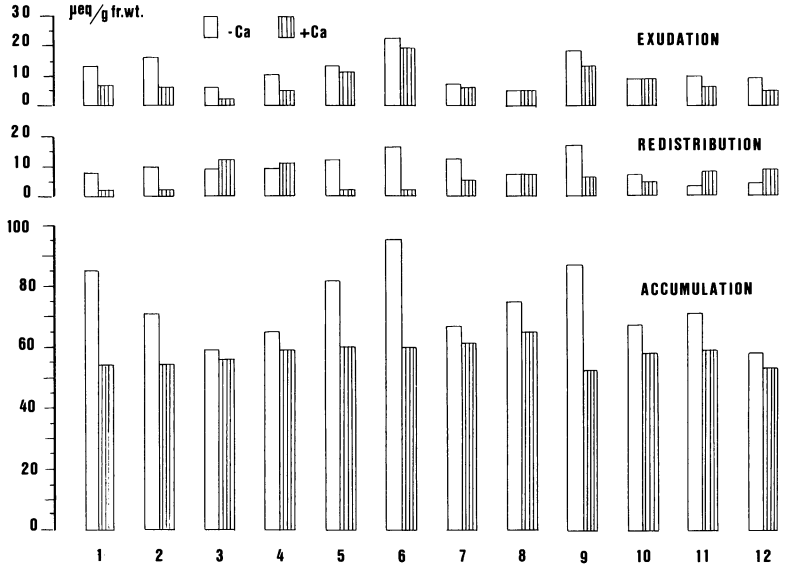

Fig. 4. Comparison between barley cultivars of their sodium accumulation, redistribution within the roots and xylem exudation, depending on the calcium presence in the outer medium.

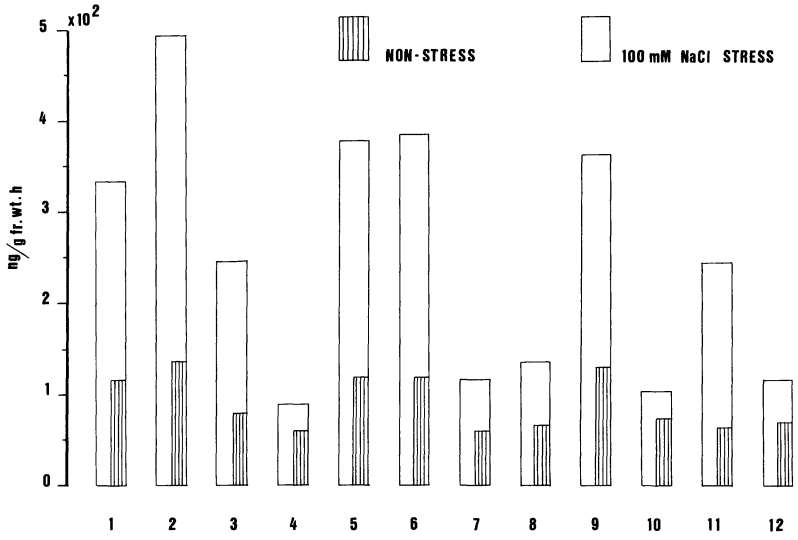

Fig. 5. Amounts of sugars transported by the phloem, from shoot to root, in non-stressed and salt stressed barley seedlings.

non-stressed plants. On average the increase was about 310% in winter barley and around 220% in summer barley, all cultivars taken into account. Within each group though some seemed more sensitive, such as Candida for winter barley and Iban for the summer barely.

Discussion

In general it was found that summer barley cultivars show a better resistance to salt-stress than winter barley cultivars. This can be partially explained due to the fact that summer barley is usually growing in a drier and warmer season than winter barley does. Physiologically though the differences are as following:

A. Due to a better ion selectivity a summer barley can withhold sodium for a longer time in the root, with most probably a better sequestration of that sodium in the vacuole. Thus less sodium is present in the symplast from where it can move to the xylem.

B. Due to better ionic-management a summer barley needs less sugar to be reclaimed from the shoot to the root, either as an energetic support or as an osmostic solute.

C. Since less sugar is required, absolute or relative, in a summer barley than in a winter barley, a summer barley can eventually out-grow the threatening stress; thus diluting the arriving sodium as more leaves will start to form. From our present findings we can select Friponne (4); Union (7) and Menuet (12) as best resisting the stress. It is remarkable though that these cultivars did perform best in long term experiment (Stassart and Bogemans, 1987), whereas Regent (3) and Iban (11) show more sensitive reactions and were classed as poor performers in the same long term experiment.

So by selecting a number of physiological parameters it is quite possible to check sensitivity to salt in short term experiments. It is our aim now to select some more measureable criteria, such as enzymatic activities, as well as ion transport and metabolic activity, to find very defined locations in the plants which can be responsible for salt-resistence or the lack of it.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 183–187.
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PLSO PN 150

Sodium exclusion mechanisms at the root surface of two maize cultivars

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Key words: membrane permeability, Na ion exclusion, salinity, *Zea mays* L.

Abstract

Sodium exclusion at the root surface of intact plants was investigated in two maize cultivars (*Zea mays* L. cv. Pioneer 3906 and cv. DeKalb XL75). The strong sodium exclusion by Pioneer 3906 relative to DeKalb XL75 was not caused by larger efflux rates. In contrast, this cultivar showed reduced influx rates resulting in about half the net uptake in comparison with DeKalb XL75. It is concluded that the cultivar difference in sodium exclusion at the root surface is based on a relatively low passive sodium permeability of the epidermal and cortex plasmalemma of Pioneer 3906. For both cultivars, the flux ratio equation indicated an active component of sodium efflux.

Introduction

Several biochemical processes in the cytoplasm of plant cells appear to be sensitive to sodium. For example, high sodium levels were inhibitory to enzymes of protein synthesis (Hall and Flowers, 1973) and glycolysis (Wiencke, 1984). Also, oxidative phosphorylation was impaired by sodium not only in isolated mitochondria of *Pisum sativum* but also in those of the halophyte *Suaeda maritima* (Flowers, 1974). In maize roots, the number of mitochondria was increased after treatment with sodium (Hecht-Buchholz *et al.*, 1971) and in beans and barley (but not sugarbeets) sodium treatment resulted in chloroplast deformation (Marschner and Mix, 1974).

Beside sodium inclusion in vacuoles exclusion of sodium ions at the plasmalemma may represent a strategy of plants to realize low cytoplasmic sodium concentrations (Flowers and Läuchli, 1983; Greenway and Munns, 1980). The latter process is especially important in root cells where ions are absorbed from the external solution. Our work with the two maize cultivars Pioneer 3906 and DeKalb XL75 revealed differences in the magnitude of sodium exclusion at the root surface (Schubert and Läuchli, 1986). Cultivar Pioneer 3906 not only excluded sodium from the shoot but also restricted sodium uptake more efficiently from roots relative to DeKalb XL75. The purpose of the present investigation was to evaluate the significance of active and passive sodium exclusion mechanisms at the root surface of these two maize cultivars.

Materials and methods

Plant cultivation

Seeds of maize (*Zea mays* L. cv. Pioneer 3906 and cv. DeKalb XL75) were imbibed in aerated 0.5 m*M* $CaSO_4$ for one day and then germinated at 27°C in the dark on filter paper moistened with 0.5 m*M* $CaSO_4$. After 2 days seedlings were transferred to a stainless steel mesh suspended over an aerated 0.5 m*M* $CaSO_4$ solution. After 2 days in this medium seedlings were used for flux experiments or further cultivated in a growth chamber until the 4th leaf stage was reached (Schubert and Läuchli, 1986).

Flux experiments with seedlings

After 2 days in 0.5 m*M* $CaSO_4$ (see 'Plant cultivation') intact seedlings were loaded with ^{22}Na ions (68 kbq ml^{-1}) for 24 h in a complete nutrient solution with the following composition: (m*M*): NaCl, 25; $CaCl_2$, 1; K_2SO_4, 0.5; NH_4NO_3, 0.75; $MgSO_4$, 0.5; (μM): NaH_2PO_4, 75; Fe-EDTA, 25; H_3BO_3, 0.5; $MnSO_4$, 0.05; $ZnSO_4$, 0.025; $CuSO_4$, 0.025; $(NH_4)_6\ Mo_{27}O_{24}$, 0.0025.

Efflux was measured after transferring the seedlings (5 replicates for each cultivar) to an identical but unlabeled 5-ml solution per seedling. This efflux medium was replaced by fresh medium after specific efflux periods (ranging from 1 minute at the beginning to 60 minutes, Fig. 1). ^{22}Na ions in the

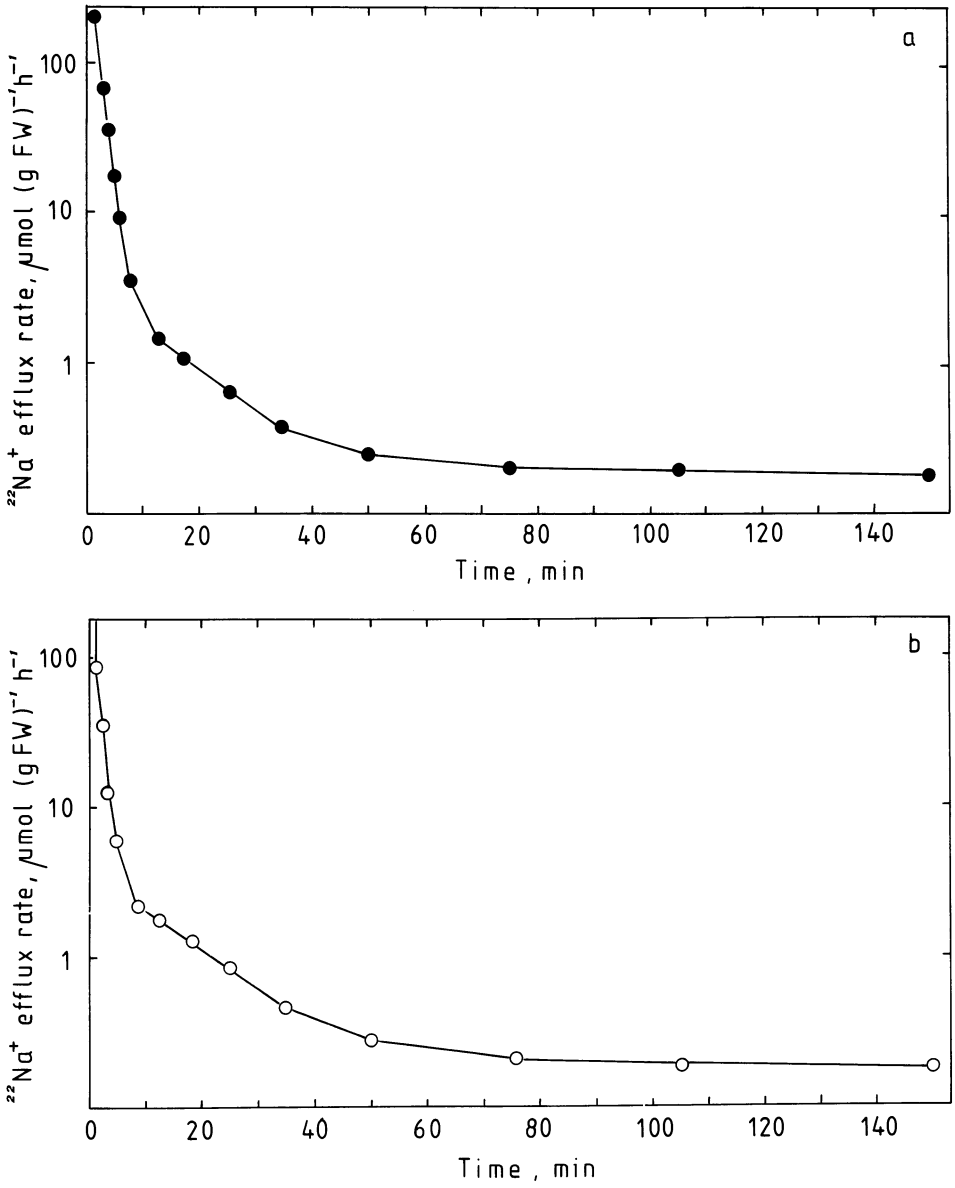

Fig. 1. Time course of ^{22}Na efflux rates of roots of intact maize seedlings (cv. Pioneer 3906 **(a)** and cv. DeKalb XL75 **(b)**) in nutrient solution. Efflux rates are given as mean values of 5 replicates for distinct efflux periods. *Each point* indicates the average efflux rate in the middle of the efflux period.

medium were determined by means of a γ-counter.

The ^{22}Na efflux rates were plotted semi-logarithmically against time (Fig. 1). To calculate the plasmalemma-controlled sodium efflux rate (J_{c0}) at the start of the experiment a linear regression of the values obtained during the period 10 to 30 minutes was calculated and extrapolated to t = 0 minutes. The cytoplasmic sodium concentration (c_c) was calculated by compartmental efflux analysis (Macklon, 1975; Pitman, 1963). The concentration c_c was calculated on the assumption that the cytoplasm comprised 10% of the root volume and that the water content was 90% of the total root weight.

Net sodium uptake from the nutrient solution was measured after preloading the seedlings in non-labeled nutrient solution as described above. After washing the roots in 10 m*M* $CaSO_4$ (24°C) for 10 minutes the root fresh weight was determined and root and shoot dry matter were analysed for sodium by flame photometry after extraction in 0.5 *M* HNO_3. Half of the plants remained in the nutrient solution for additional 24 hours and were then treated in the same way. From these two harvests the net sodium uptake rate was calculated.

Determination of net sodium uptake at the 4th leaf stage

At the 4th leaf stage plants were transferred to a 4 × concentrated nutrient solution containing 25 m*M* NaCl and after 12 h to a 4 × nutrient solution containing 50 m*M* NaCl. After 48 h in this medium (= day 1) one third of the plants (4 plants for each of the 3 replicates) was harvested, divided into roots and shoots, and the fresh and dry matter were determined. Sodium in the ground plant tissue was analysed as described for seedlings. This procedure was repeated after 24 h (day 2) and 48 h (day 3) NaCl treatment. The data of these three harvests allowed us to calculate net sodium uptake rates between day 1 and 2 as well as between 2 and 3. The mean of these two rates was then calculated.

Determination of sodium efflux at the 4th leaf stage

Plants at the 4th leaf stage were preloaded with 25 and 50 m*M* NaCl, respectively, as described for the uptake experiments. After this pretreatment roots of intact plants were washed in 10 m*M* $CaSO_4$ (24°C) for 10 minutes and, subsequently, sodium efflux into 4 m*M* $CaCl_2$ (220 ml per plant) was measured by flame photometry at 3 h intervals. Since efflux rates represented average values over 3 h intervals the efflux rates are given for the middle of each efflux period, *e.g.* after 1.5 h for the first interval (Fig. 2).

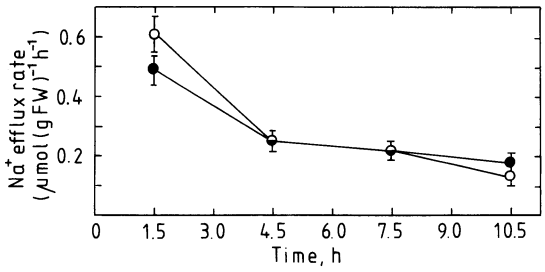

Fig. 2. Time course of sodium efflux rates of roots of intact maize plants at the 4th leaf stage (cv. Pioneer 3906 (●) and cv. DeKalb XL75 (○)) in 4 m*M* $CaCl_2$. Efflux rates are given as mean values of 6 independent experiments, each with 4 replicates, for distinct efflux periods. *Each point* indicates the average efflux rate in the middle of the efflux period. Efflux started at t = 0. *Vertical bars* indicate the standard error.

Membrane potential measurements

Membrane potentials were measured in root cells of intact seedlings after 24 h in nutrient solution. Seedlings were mounted in a Plexiglas chamber allowing the fixed root to be immersed in the test solution. Membrane potentials in the meristematic, extension, and root hair zone were recorded by standard electro-physiological means as described by Ullrich-Eberius *et al.* (1983). Membrane potentials did not differ significantly for the various root zones.

Results

In seedlings, ^{22}Na efflux J_{c0} from DeKalb XL75 was slightly but not significantly higher compared with that from Pioneer 3906 (Table 1). Furthermore, net sodium uptake was almost twice as high in DeKalb XL75 relative to Pioneer 3906 (P = 1%), a result found earlier in long term

Table 1. Sodium influx and efflux rates of roots of intact maize seedlings (cv. Pioneer 3906 and cv. DeKalb XL75). Flux rates were determined in nutrient solution as described in 'Materials and methods'

Flux	Flux rates (μmol Na $(g\,FW)^{-1}\,h^{-1}$)	
	Pioneer 3906	DeKalb XL75
Net sodium uptake, measured	2.43 ($\pm$0.27)	4.80 ($\pm$0.84)
^{22}Na efflux, measured	3.31 ($\pm$0.26)	3.45 ($\pm$0.80)
Sodium influx, calculated	5.74	8.25

greenhouse experiments (Schubert and Läuchli, 1986). Apparently, low net uptake in Pioneer 3906 was not due to larger efflux rates (J_{c0}) but was caused by reduced influx rates (J_{c0}) (Table 1).

Since during plant ontogeny salt tolerance of maize changes with age (Maas, 1984) we determined sodium efflux also at the 4th leaf stage. After washing the roots in 10 m*M* $CaSO_4$ for 10 minutes cell wall sodium is likely to have been washed out. Therefore, during the initial 3 h efflux was limited by the plasmalemma and the tonoplast. The efflux curves for the two cultivars showed no differences in efflux rates after 3 h (Fig. 2). In contrast, during the initial 3 h sodium efflux from DeKalb XL75 was slightly higher (P = 5%) compared with that from Pioneer 3906 (Fig. 2, Table 2). As there was no cultivar difference between vacuolar efflux rates the initial higher efflux rate of DeKalb XL75 has to be ascribed to plasmalemma-controlled efflux and reinforces our suggestion that low net sodium uptake in Pioneer 3906 (Tables 1 and 2) is a consequence of low influx rates (J_{0c}) in this cultivar.

Membrane potential measurements with seedlings showed no significant differences between the two cultivars (Table 3). Table 3 also gives a summary of flux ratios (J_{0c}/J_{c0}), cytoplasmic (c_c) and external (c_0) sodium concentrations, as well as predicted flux ratios calculated from the Ussing/Teorell equation.

Discussion

The measured flux ratios proved to be considerably lower than the predicted values (Table 3). An active sodium efflux component (against an electrochemical gradient) is therefore indicated for both cultivars. This result is in agreement with findings of Davis and Jaworski (1979).

The cultivar difference in sodium exclusion (Tables 1 and 2), however, was not related to larger efflux (active exclusion) since efflux rates (J_{c0}) were equal or even smaller in Pioneer 3906 relative to DeKalb XL75 (Tables 1 and 2). Influx rates (J_{c0}),

Table 2. Sodium net uptake and efflux rates of roots of intact maize plants at the 4th leaf stage (cv. Pioneer 3906 and cv. DeKalb XL75). Net uptake was measured in 4 $\times$ nutrient solution including 50 m*M* NaCl. Efflux during 3 h was determined in 4.0 m*M* $CaCl_2$ as described in 'Materials and methods'

Flux	Flux rates (μmol Na $(g\,FW)^{-1}\,h^{-1}$)	
	Pioneer 3906	DeKalb XL75
Net Na uptake	0.50 ($\pm$0.06)	1.05 ($\pm$0.10)
Na efflux	0.54 ($\pm$0.01)	0.67 ($\pm$0.03)

Table 3. Sodium influx/efflux ratios (J_{0c}/J_{c0}), cytoplasmic (c_c) and external (c_0) sodium concentrations, membrane potentials (E_m) and predicted flux ratios calculated from the Ussing/Teorell equation $J_{0c}/J_{c0} = c_0/c_i\,e^{(zFE/RT)}$ for roots of intact maize seedlings (cv. Pioneer 3906 and cv. DeKalb XL75)

Parameter	Pioneer 3906	DeKalb XL75
J_{0c}/J_{c0}, measured	1.73	2.28
c_c (m*M*)	9.41	11.00
c_0 (m*M*)	25.00	25.00
E_m (mV)	$-122(\pm 5.1)$	$-118(\pm 8.6)$
$c_0/c_c\,e^{(zFE/RT)}$, calculated	299	220

on the other hand, were higher in DeKalb XL75 (Table 1). From these results we conclude that the cultivar difference in sodium exclusion at the root surface results from a lower passive sodium permeability of the plasmalemma of epidermal and cortex cells in Pioneer 3906 relative to DeKalb XL75.

Although a simple positive correlation between sodium exclusion and salt tolerance has not been observed (Lessani and Marschner, 1978), this property of Pioneer 3906 may be of significance to plant breeders who select for salt tolerance. The passive exclusion mechanism enables the plant to exclude sodium without a permanent expenditure of energy which would be necessary for an active mechanism. Breeding for salt tolerance, however, seems to require an improvement of osmotic adjustment as well (Schubert and Läuchli, 1986).

Net sodium uptake was considerably larger for seedlings than for plants at the 4th leaf stage (Tables 1 and 2). With a higher Ca/Na ratio in the medium, a factor that might have reduced the root permeability for Na ions (Greenway and Munns, 1980; La Haye and Epstein, 1969), seedlings showed poorer sodium exclusion capabilities than older plants. This may in part explain the extreme salt sensitivity of maize during the seedling stage (Maas *et al.*, 1983). It is possible that the plasmalemma of seedlings exhibits a higher sodium permeability. On the other hand, sodium absorption could be confined to specific regions of the root system, for instance young root zones that comprise a higher proportion in seedlings.

Acknowledgements

The authors thank Dr C I Ullrich-Eberius for the opportunity to carry out electro-physiological measurements at the Institute of Botany, Technical University Darmstadt, FRG. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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PLSO PN 151

Cation accumulation related to adaptation of maize populations to salinity

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Key words: adaptation, ionic balance, maize, populations, salinity

Abstract

Maize populations collected from various biotopes which differ in their degree of salinity were grown in agrolite with a nutrient solution containing 0, 20, 40, 60 and 80 mN of NaCl, for 15 days. The growth data show a wide phenotypic plasticity, and the cationic accumulation at 80 mN suggests an interesting nutritional differentiation within maize populations. The results show that the progeny of seeds collected from the non-saline soils, accumulate proportionally more sodium and less magnesium within their tissues than those populations from the saline biotopes. Furthermore the lowest accumulation of sodium and the largest accumulation of magnesium were found on populations that were genetically isolated; that is on the progeny of seeds collected from biotopes where only autopollination or pollination with plants tolerant to salts was possible. An intermediate behavior upon sodium and magnesium accumulation, was observed in the progeny of seeds collected from plants grown in the saline soils, surrounded by maize plants grown in a less saline condition. The results suggest a differential cation accumulation within maize populations that is genetically controlled. The exclusion of sodium from the tissues and, or the increased accumulation of magnesium seemed to be related to a maize adaptation strategy to salinity.

Introduction

Maize is a grain crop considered unable to survive under saline conditions. Nevertheless, in the region near the ancient border of the ex-lake of Texcoco (Mexico), maize is the predominant culture. The salinity in this region is very high in some sites and frequently maize grows associated with *Suaeda nigra*, a facultative halophyte. The surviving amplitude observed within maizes, could be related to a wide phenotypic plasticity within the species or to a more or less pronunciated physiological differentiation.

The cationic (Ca, Mg, K and Na) accumulation in tissues (Baus, 1986; Wacquant and Bouab, 1983) or in the xylem sap (Wacquant *et al.*, 1981) has been used to prove the existence of an intraspecific nutritional differentiation between plants that naturally occurs in different soil conditions. In order to test if the presence of maize in saline and non-saline biotopes is related to a physiological differentiation, the cationic accumulation in the tissues from maize populations was assayed.

Materials and methods

The seeds employed in the experiment came from the basal part of the maize ear (8 or 12 ears per site) and were collected from ten habitats with different salinity degree. Prior to threshing the ears and forming a composite sample per site, the races of maize were identified. The sites (2, 4, 5, 6, 10 and 11) were saline and the rest (17, 19, 20 and 21) non saline. The saline habitats 4, 10 and 11 were isolated genetically. That is, the seeds belong to plants growing in a biotype where only autopolination or polination within maizes tolerant to salinity was possible. On the other hand, the seeds collected in the saline habitats 2, 5 and 6 came from plants growing on a saline soil, surrounded by plants developing in a less saline condition. The last was

assumed because of better development of the plants and the absence of *Suaeda nigra* between the rows.

The edaphic characteristics (pH and E.C.) were determined in the water extract of a soil sample (0 to 15 cm) taken in the site where the seeds were collected.

The experimental procedure comprised the growth, in duplicate, of 5 plants of each habitat in a pot (250 ml) filled with agrolite. During the germination period and all over the experiment the pots were irrigated twice a day with a nutrient solution containing 0, 20, 40, 60 and 80 m*N* of NaCl. The composition of the nutrient solution was 1.0, 0.4, 0.4 and 0.2 m*N* of $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KH_2PO_4 and 0.73, 1.82, 0.16, 0.01, 0.61 and 2.83 m*M* of Mn, B, Ca, Mo, Zn and Fe. Twelve days after germination the plants were cut and the fresh and dry weight of tops recorded.

The content of Ca, Mg, K and Na was determined after digestion of the tops (5 per pot) with H_2SO_4 and H_2O_2 at 200°C. Cations were determined by flame emission (Ca, K and Na) and atomic absorption spectrophotometry (Mg).

Results

Table 1 shows that the salinity is very high at sites 6, 10 and 11. Even though this does not imply that plants in this biotope have grown or tolerated this salinity level all over their cycle. The high values recorded could be associated with salt accumulation at the soil surface caused by a capillarity process during a dry period; the samples were taken two months after the end of the rainy season. Sites 2, 4 and 5 exhibit a lower salinity, as compared to the other saline sites, but a higher pH, probably due to the presence of carbonates and bicarbonates (Lindsay, 1979) in the soil system. In contrast with the former habitats described, sites 17, 19, 20 and 21 are neutral and non-saline according with the pH and E.C. values shown.

Table 1. Edaphic characteristics (pH and E.C.), water management and maize-race prevailing in the different sites

Site	pH	E.C. $d\,Sm^{-1}$	Water management	Race
2	9.1 ± 0.2	2.9 ± 0.1	NI	C
4	9.1 ± 0.1	2.8 ± 0.2	NI	C
5	9.1 ± 0.3	6.6 ± 0.2	NI	C
6	8.6 ± 0.1	28.3 ± 5.9	NI	C
10	8.4 ± 0.2	110.0 ± 15.5	Ni	C
11	8.6 ± 0.2	33.0 ± 8.0	NI	C
17	7.0 ± 0.1	0.8 ± 0.1	NI	C
19	7.2 ± 0.1	0.4 ± 0.1	I	C.Ch
20	7.2 ± 0.1	0.4 ± 0.1	I	C.Ch
21	7.2 ± 0.2	0.4 ± 0.1	I	C.Ch

NI, not irrigated; I, irrigated; C, Conico; C.Ch, cross-breeding between conico and Chalqueño.

On the other hand, Table 1 shows that the Conico maize race prevails in the not irrigated biotopes and the cross breeding between Conico and Chalqueño is dominant on the irrigated soils.

Growth data show that the mean percentage of growth restriction with respect to the 0.0 m*N* treatment was, at 80 m*N*, 47.21 ± 7.0 for the saline populations 4, 10 and 11 (populations isolated); 44.45 ± 7.6 for the saline populations 2, 5 and 6 (populations not isolated) and 44.66 ± 1.8 for the cross breeding populations coming from the non saline sites (19, 20 and 21). At 20, 40 and 60 m*N* the percentage of growth restriction was between 10 and 30% for all the populations and no difference within the populations was observed either. The former values shown are comparable to those obtained with maize cv D.C. 790 for almost the same range of NaCl concentrations (Marschner, 1986).

In contrast with the growth parameters, cation accumulation in the tissues shows notable differences between maize populations at 80 m*N*. This was not observed at the other saline treatments (0, 20, 40 and 60 m*N*). Figure 1 compares the cationic accumulation within maize populations at 80 and 0.0 m*N* of NaCl. The results show that at 80 m*N* the progeny of populations collected in the non-saline biotopes (17, 19, 20 and 21) accumulates proportionally more sodium and less calcium and magnesium than those populations collected on the saline biotopes. Furthermore, the lower accumulation of sodium and the greater accumulation of divalent cations was found on populations that were genetically isolated (4, 10 and 11). That is, within the progeny of seeds collected from biotopes where only autopolination or polination between tolerant maize plants to salt was possible. An intermediate behavior upon sodium and divalents accumulation, was observed within the progeny of seeds collected from plants grown in the saline sites

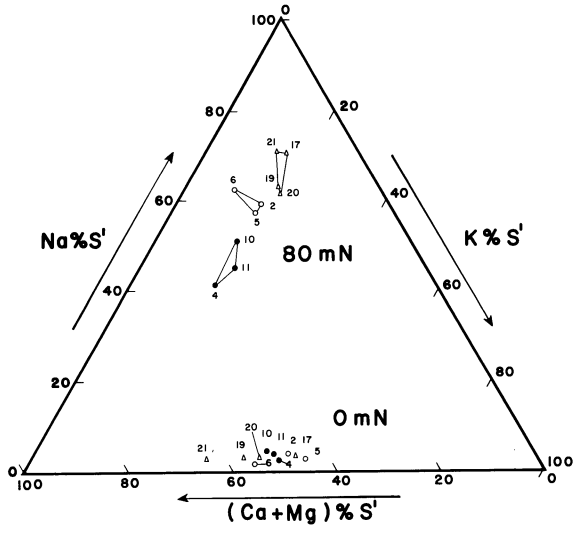

Fig. 1. Proportions of Na, K and Ca + Mg%S′ in the tops of 10 maize populations at 0 and 80 mN NaCl. S′ = Sum (Na, K, Ca and Mg) in mN g^{-1} dry weight. (△) none saline populations, (○) saline populations; not isolated, (●) saline populations; isolated.

(2, 5 and 6), that were surrounded by plants grown in a less saline condition.

In absence of added NaCl no differentiation between groups was observed. Nevertheless, the cross-breeding progeny of sites 19, 20 and 21 shows a slight tendency to accumulate more divalents, while the opposite behavior was observed for the same genotypes, at 80 m*N*.

The differences among populations on the divalent accumulation at 80 m*N* are rather due to the magnesium accumulation. Table 2 shows that the calcium and magnesium accumulation within populations, ranges in terms of the Mg%S′ between 13.1 and 46.2% and that of Ca%S′ between 1.0 and 2.8%. Also the Mg%S′ (Table 2) suggests a trend of the saline populations to accumulate more magnesium than the non-saline ones. The increase of the magnesium accumulation, as a response to the presence of NaCl in the nutrient solution, has been reported to occur in the roots of *Inula critmoïdes*, an halophilic species (Ballo, 1985).

The variation for the Na, K, Ca and Mg percentages (Table 2) shows a remarkable stability within the cross-breeding (C·Ch) populations 19, 20 and 21. This situation was not the same for the Conico populations (six natives from saline biotopes and one from a non-saline habitat). The greater variation within the Conico populations, possibly reflects a lower degree of genetic homogeneity of those populations in contrast with the Conico and Chalqueño cross breeding populations. Simultaneously it is interesting to note that for saline populations the lower variation of the %NaS′ is associated with the populations genetically isolated (4, 10 and 11). The opposite occurs for the %KS′ and no clear relationships can be established for the Ca%S′ and Mg%S′. The relative higher variation of the Na%S′ and Mg%S′ within the populations natives from the saline biotopes, supports the idea that the accumulation of magnesium occurs to the detriment of that of sodium or *vice versa*. The latter is shown in Figure 2, where the Na%S′ is plotted against the Mg, Ca and K (%S′). In this figure, we can observe that when the Na%S′ increases in the tissues the Mg%S′ decreases and that no significative relationship was appreciated between the Na%S′ and the Ca and K (%S′).

Table 2. Proportions of Na, K, Ca and Mg%S′ accumulated in the tops of maize populations at 80 m*N* NaCl

Population	Na%S′	K%S′	Ca%S′	Mg%S′
2	34.5 ± 23.6	17.5 ± 1.8	1.9 ± 0.8	46.2 ± 21.0
4	40.1 ± 3.5	11.8 ± 4.7	2.8 ± 1.3	45.1 ± 9.7
5	57.1 ± 10.3	15.6 ± 1.1	1.8 ± 0.4	25.1 ± 11.1
6	62.0 ± 12.0	9.0 ± 1.4	1.0 ± 0.3	27.8 ± 10.9
10	50.4 ± 6.6	15.2 ± 5.1	1.2 ± 0.6	33.0 ± 25.2
11	44.4 ± 3.0	18.0 ± 10.9	1.2 ± 0.4	36.2 ± 14.4
17	70.0 ± 8.7	14.9 ± 0.6	2.0 ± 0.2	13.1 ± 8.3
19	62.6 ± 0.6	17.4 ± 0.1	2.5 ± 0.1	17.4 ± 0.9
20	61.6 ± 0.6	17.6 ± 1.7	2.0 ± 0.2	18.9 ± 0.8
21	70.6 ± 0.6	13.9 ± 0.5	1.9 ± 0.1	13.5 ± 1.2

S′ = Sum of Na, K, Ca and Mg in m*N* g^{-1} dry weight.

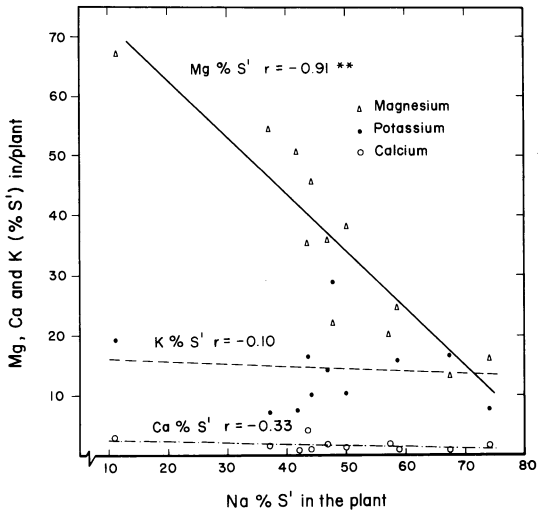

Fig. 2. Relationships between the equivalent percentage of Na (Na%S') and those of Mg, Ca and K (%S'), obtained in the tissues of ten maize populations at 80 mN NaCl.

Discussion and conclusion

Growth data failed to demonstrate a differentiation within the maize populations and suggest a wide phenotypic plasticity for the maize populations in the region. In contrast cation accumulation shows an interesting nutritional differentiation.

The same has been reported to occur in other species (Baus, 1986; Wacquant *et al.*, 1981; Wacquant and Bouab, 1983). The exclusion of sodium and or the greater accumulation of magnesium in the tissues at 80 m*N*, seems to be the principal characteristic of the progeny of seeds collected in the saline and isolated habitats. According to Greenway and Munns (1980) and Pitman (1984) many non halophytic species avoid or exclude sodium and chlorides from their tissues, as an adaptative choice to salty conditions. The sodium exclusion observed in our case is correlated with a higher accumulation of magnesium. Similar increases on magnesium accumulation have been reported to occur in the roots of *Inula critmoïdes*, an halophilic species (Ballo, 1985) and in the xylem sap of *Dittrichia viscosa* an ubiquitous brush (Baus, 1986), as a response to high NaCl concentrations in the nutrient solution. The role of the enhanced accumulation of magnesium is not clear. Even though it is interesting to note according to Sánchez and Azuara (1980), that the magnesium sulfate treatment produces a lower fresh and dry weight restriction with respect to the control as compared to PEG-4000 at the same osmotic pressure. Hence, the higher magnesium affinity observed in this group could be of adaptative significance.

The separation of groups at 80 m*N* (Fig. 1) according to their edaphic origin and cross breeding relationships prevailing in their habitats, suggest a nutritional differentiation that is genetically controlled. The last is emphasized by the intermediate behavior observed (cationic accumulation) by the progeny of seeds collected from plants grown on the salty soils surrounded by plants growing in a less saline habitat. The lower variation shown in the cationic accumulation and in the dry weight percentages within the Conico and Chalqueño cross breeding populations, is possibly related to the more generalized use of corn inbreeds on the irrigated soils. Contrastingly, on the non-irrigated soils this practice is almost inexistent. As a conclusion, this work did not show any significative growth difference, among the progeny of ten maize populations, when they are compared under the same growing conditions. However, it provided evidence for a nutritional differentiation of adaptative significance. The progeny of seeds from the saline and isolated habitats excludes sodium from their tissues and accumulate more magnesium, whereas the progeny of seeds from the non-saline soil do not control the accumulation of sodium. The apparent indifference of maize to saline conditions should be explained at least partially by a genetic differentiation in the ability of plants to select ions.

Acknowledgement

Thanks are due to Mrs Carolina Moreno Rodríguez for her technical assistance.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 195–202.
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PLSO PN 152

Interaction between nitrogen and phosphorus fertilizers and soil salinity and its effect on growth and ionic composition of corn (*Zea mays* L.)

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Key words: soil salinity, soil salinity-fertilizer interactions, *Zea mays*

Abstract

A pot experiment was conducted to study the interaction between soil salinity and two major nutrients, nitrogen and phosphorus, and the effect of this interaction on the growth and ionic composition of corn plants.

Four levels of soil salinity, 3.4, 6.7, 9.2, and 12.5 ds m^{-1} were prepared and four rates of nitrogen, equivalent to 0, 120, 180, and 240 kg N ha^{-1}, and four rates of phosphorus, equivalent to 0, 60, 120, and 180 kg P_2O_5 ha^{-1}, were added separately and combined. A factorial completely randomized design with three replicates was used. At the flowering stage, the plants were harvested and separated into stems, leaves and roots. Nitrogen, phosphorus, potassium, calcium, magnesium, sodium and chloride were determined in the plant parts.

The results showed that increasing soil salinity caused a decrease in dry matter, and an increase in the concentration of nitrogen, sodium, magnesium and chloride in most plant parts.

Application of nitrogen and phosphorus fertilizers under saline conditions improved the growth of corn and reduced the concentration of chloride and magnesium.

The results also indicated that adding nitrogen and phosphorus fertilizers together, produced better positive interaction with salinity than when added separately. Applications of nitrogen and phosphorus in quantities equivalent to 240 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1} were found to be the optimum levels for corn under the salinity levels used in this experiment.

Introduction

The extensive occurrence of salt-affected soils in the Mesopotamian plain is one of the main problems of Iraqi agriculture (Al Taie, 1970; Buringh, 1960). Soil salinity, as reported by many investigators (Bernestein, 1975; Bressler *et al.*, 1982; Mass and Hoffman, 1977), affects plant growth by one or more of the following factors, osmotic effect, toxic effect, disturbance of nutritional ion balance and indirect effects induced by the adverse properties of saline soils.

The results of some experiments done on various crops growing in different saline media (Al Rahwi and Sadallah, 1980; Amer *et al.*, 1964; Finck, 1976; Humadi, 1977; Lale and Singh, 1973) showed that adding chemical fertilizers may reduce the salinity effects on plant growth and consequently improve plant production in some saline soils.

The purpose of this work was to study the interaction between nitrogen and phosphorus fertilizers and soil salinity in respect of the growth and the chemical composition of corn.

Materials and methods

A pot experiment was conducted for this study, with eight kilograms of soil in each pot. The bulk sample of soil used was collected from the surface layer (0—30 cm) of the fields of the College of Agriculture, University of Baghdad. The main

characteristics of the soil were, texture, clay silt loam; Organic matter, 0.99 %; $CaCO_3$, 22.0 %; Cation exchange capacity, 18.2 Cmol kg^{-1}; ECe, 4.1 dSm^{-1}; pH, 8.1.

Four soil salinity levels 3.4, 6.7, 9.7 and 12.5 dSm^{-1}, were prepared by leaching the soil in the pots with water having the required salinity level, until the drainage water reached equilibrium with the leaching water. The soil salinity levels were designated in this paper as S_1, S_2, S_3 and S_4, respectively. Four nitrogen levels, 0, 120, 180 and 240 kg N ha^{-1} as urea, designated as N_0, N_1, N_2 and N_3, respectively, and four levels of phosphorus, 0, 60, 120 and 180 kg P_2O_5 ha^{-1} designated as P_0, P_1, P_2 and P_3, were added separately and combined to the salinized soil in the pots. Each soil pot received in addition, the equivalent of 60 kg K ha^{-1} of basic fertilizer as potassium sulfate. Ten seeds of corn (*Zea mays* L. hybrid R-1) were sown in each pot and after 10 days the seedlings were thinned to 6 plants. Soil pots were irrigated when soil losses amounted to 50—60 % of the available water.

Immediately after the flowering stage the plants were harvested and separated into stems, leaves and roots, and then dried. Nitrogen, phosphorus, potassium, calcium, magnesium, sodium and chloride were determined in the plant parts. Soil analysis was carried out according to the standard procedures of Black (1965) and plant analysis according to the methods described by Chapman and Parker (1961). A factorial completely randomized block design, with three replicates, was used in this experiment. Statistical analysis was by the methods described by Steel and Torrie (1960).

Results

Dry matter

The effect of different soil salinity levels (with and without fertilizer application) on the dry matter weight of the leaves, stems and roots are shown in Figure 1. These data showed that the dry matter weight of these parts significantly decreased as soil salinity increased (treatments S_1, S_2, S_3, and S_4). However, the average value of reduction in the dry matter weight of the roots, due to the soil salinity increase, was more than that of the upper parts of the plant.

Application of increasing doses of nitrogen fertilizer (treatments N_0P_0, N_1P_0, N_2P_0 and N_3P_0) caused an increase of the dry matter weight of all plant parts, particularly the dry matter weight of the aerial parts, at all soil salinity levels (Figure 1B). However, the best positive interaction between applied nitrogen fertilizer and soil salinity, in this respect, was found in the treatment S_2N_1. Applica-

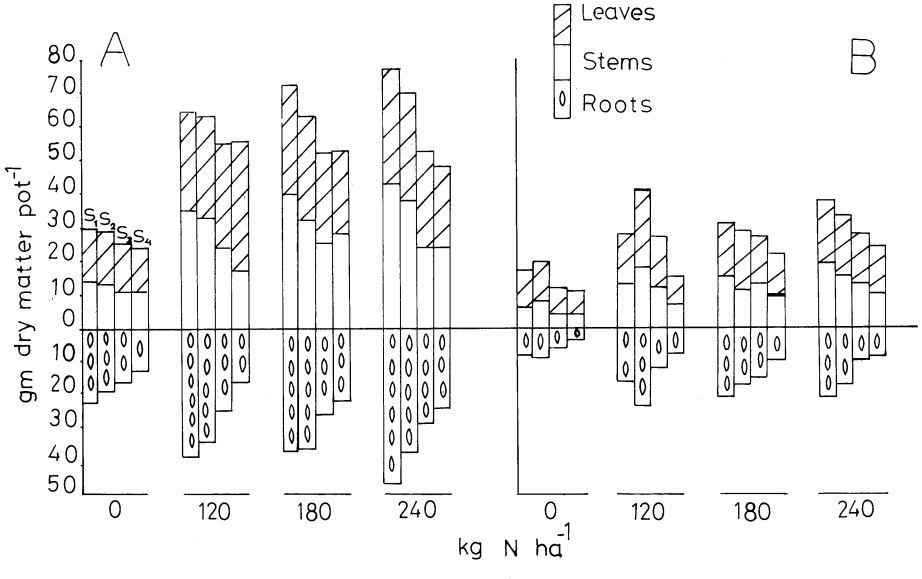

Fig. 1. Interactive effect of soil salinity and nitrogen fertilizer on the dry matter of corn. **A**. Nitrogen and phosphorus, **B**. Nitrogen.

tion of nitrogen together with phosphorus caused higher increments in the dry matter weight of the plant parts of all soil salinity levels (Fig. 1A). A significant increase in the dry matter weight of the plant parts at all soil salinity levels was also found when phosphorus fertilizer alone was applied (Fig. 2B). However, a higher increase in the dry matter was observed in roots in comparison with the upper parts. The best interaction between applied phosphorus fertilizer and soil salinity was found in treatment S_1P_3. Application of phosphorus together with nitrogen caused a higher increase in the dry matter weight of all plant parts at all soil salinity levels (Fig. 2A)

Nitrogen

Figure 3 shows that increasing soil salinity levels caused a significant increase of nitrogen concentration in the leaves, stems and roots of corn. When increasing doses of nitrogen were applied, the nitrogen concentration increased significantly in all plant parts at all soil salinity levels (Fig. 3B). The highest nitrogen concentration was found in the treatment S_4N_3. Application of nitrogen fertilizer together with phosphorus fertilizer also caused an increase in the concentration of nitrogen in all plant parts, however, the nitrogen concentration was found to be lower than when nitrogen fertilizer was applied alone. When increasing doses of phosphorus fertilizer were applied alone, a significant decrease in nitrogen concentration was found in all plant parts at all soil salinity levels. When phosphorus was applied together with nitrogen, a decrease in nitrogen concentration was also observed in the plant parts, but the levels of nitrogen were relatively higher than those found when phosphorus fertilizer was applied alone.

Phosphorus

As it is shown in Table 1 that phosphorus concentration in all plant parts decreased significantly with increased soil salinity so also, when increasing doses of nitrogen fertilizer were applied, a decrease in phosphorus concentration was also recorded in all plant parts and at all soil salinity levels. The lowest concentration level of phosphorus was found in treatment S_4N_3: when nitrogen fertilizer was applied together with phosphorus fertilizer, the general trend mentioned above in respect of phosphorus concentration was not affected. However, the levels of phosphorus in plant parts were relatively higher in comparison with those

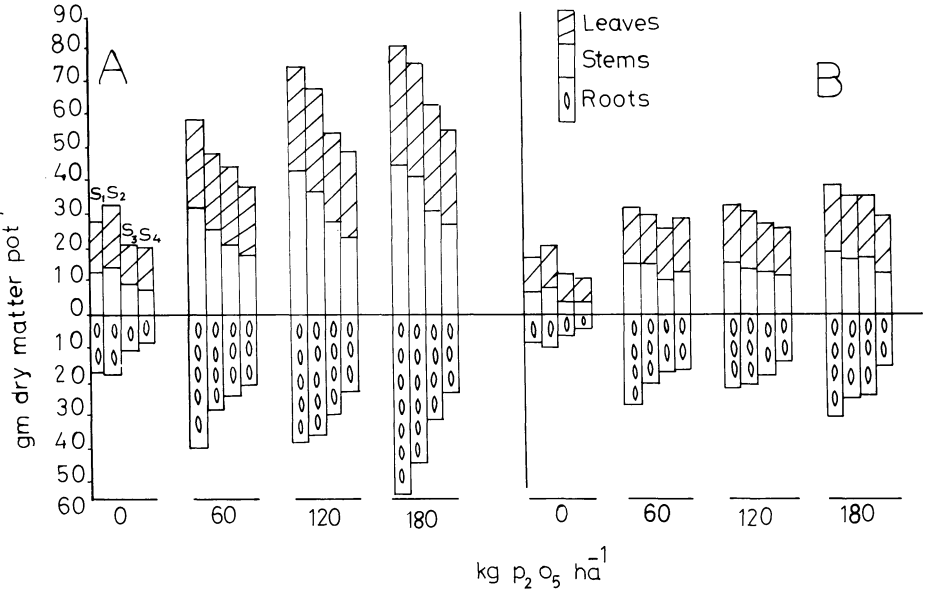

Fig. 2. Interactive effect of soil salinity and phosphorus fertilizer on the dry matter of corn. **A**. Phosphorus and nitrogen, **B**. Phosphorus.

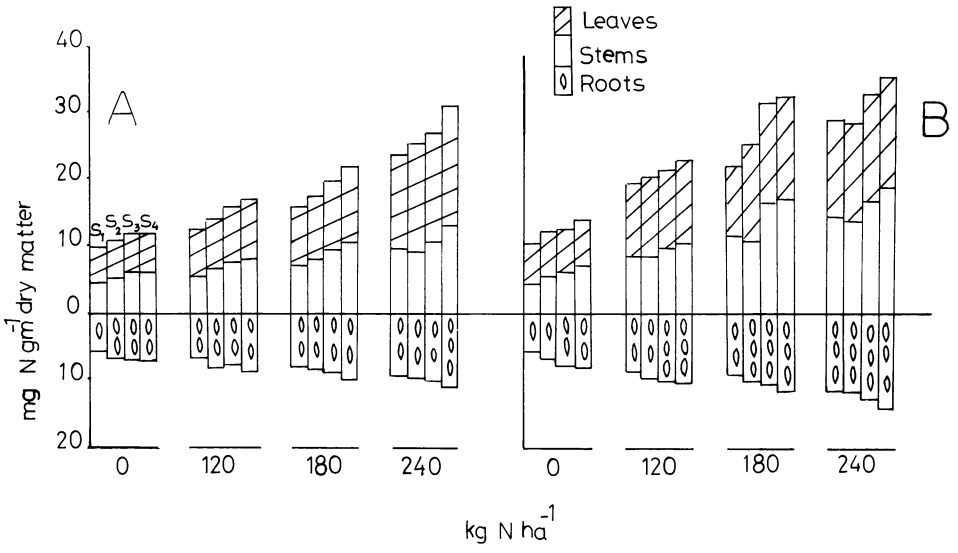

Fig. 3. Interactive effect of soil salinity and nitrogen fertilizer on nitrogen concentration in the plant parts. **A**. Nitrogen and phosphorus, **B**. Nitrogen.

Table 1. Interactive effect of soil salinity and nitrogen fertilizer on phosphorus concentration (mg g^{-1} dry matter) in the plant parts

Soil salinity (dS m^{-1})	Level of nitrogen fertilizer (kg N ha^{-1})				Mean	L.S.D$_{0.05}$
	0	120	180	240		
Leaves						
3.4	4.00	3.39	3.17	3.10	3.42	N × S = 0.19
6.7	3.71	3.24	3.08	2.79	3.21	N = 0.13
9.2	3.43	3.14	3.05	2.70	3.08	S = 0.13
12.5	3.22	2.93	2.85	2.64	2.91	
Mean	3.59	3.18	3.04	2.81	3.16	
Stems						
3.4	4.92	4.03	3.67	3.98	4.15	N × S = 0.31
6.7	4.23	3.79	3.42	3.07	3.63	M = 0.22
9.2	4.20	3.33	3.90	2.95	3.41	S = 0.22
12.5	3.92	3.19	2.93	2.82	3.21	
Mean	4.31	3.58	3.30	3.21	3.60	
Roots						
3.4	5.32	4.68	4.68	4.03	4.68	N × S = not
6.7	4.96	4.45	4.16	3.86	4.36	significant
9.2	4.82	4.27	3.88	3.78	4.19	N = 0.32
12.5	4.48	4.36	4.13	3.84	4.31	
Mean	4.89	4.36	4.13	3.84	4.31	

N = L.S.D between nitrogen fertilizer levels.
S = L.S.D between salinity levels.
N × S = L.S.D between treatments.

found in the treatments where nitrogen fertilizer was applied alone.

Application of phosphorus fertilizer alone caused a significant increase of phosphorus concentration in all plant parts at all soil salinity levels (Table 2). The best positive interaction between applied phosphorus fertilizer with soil salinity in this respect was noticed in the treatment S_1P_3. When phosphorus fertilizer was applied together with nitrogen fertilizer, an increase in phosphorus concentration in all plant parts was also found but with less significance.

Potassium, calcium, magnesium, sodium and chloride

Data concerning the effect of soil salinity on the concentration of potassium, calcium, magnesium, sodium and chloride in the corn plant parts are summarized in Figure 4. These data show that the increase of soil salinity caused a significant decrease of potassium and calcium concentration and a significant increase of magnesium, sodium and chloride concentration in the plant parts. Application of increasing doses of nitrogen fertilizer caused, in general, a decrease of potassium and calcium concentration in the plant parts at all soil

Table 2. Interactive effect of soil salinity and phosphorus fertilizer on phosphorus concentration ($mg\,g^{-1}$ dry matter) in the plant parts

Soil salinity	Level of phosphorus fertilizer ($kg\ P_2O_5\ ha^{-1}$)				Mean	L.S.D 0.05
(dSm^{-1})	0	60	120	180		
Leaves						
3.4	3.37	3.74	4.00	4.93	4.01	S × P = 0.25
6.7	3.27	3.53	3.93	4.10	3.72	P = 0.13
9.2	3.11	3.28	3.58	3.75	3.43	S = 0.13
12.5	2.72	2.26	3.31	3.63	2.98	
Mean	3.12	3.20	3.71	4.10	3.34	
Stems						
3.4	4.16	4.43	5.39	5.69	4.92	S × P = not
6.7	3.68	4.05	4.49	4.69	4.22	significant
9.2	3.58	4.07	4.46	4.68	4.20	P = 0.18
12.5	3.43	3.56	4.04	4.64	3.92	S = 0.18
Mean	3.71	4.03	4.60	4.93	4.32	
Roots						
3.4	4.76	5.20	5.43	5.88	5.32	S × P = not
6.7	4.36	4.49	5.40	5.56	4.95	significant
9.2	4.44	4.76	5.11	3.22	4.88	P = 0.47
12.5	4.28	4.00	4.78	4.88	4.86	S = 0.47
Mean	4.46	4.61	5.18	5.39	4.91	

P = L.S.D between phosphorus fertilizer levels
S = L.S.D between salinity levels.
P × S = L.S.D between treatments.

salinity levels. The lowest potassium concentration values in the leaves, stems and roots were found in the treatments S_3N_3, S_4N_2 and S_4N_3, respectively. While the lowest calcium concentration values in the leaves, stems and roots were found in the treatments S_4N_3, S_3N_3 and S_4N_3, respectively. Nitrogen fertilizer applied together with phosphorus fertilizer also caused a decrease of potassium and calcium concentration in all plant parts at all soil salinity levels. Phosphorus fertilizer application caused a positive interaction with soil salinity in respect of potassium concentration in the plant, in particular, in the plant roots.

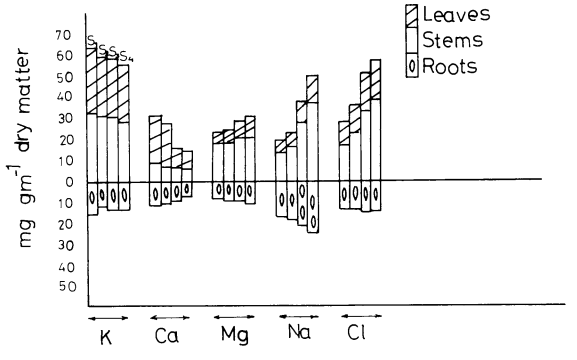

Fig. 4. Effect of soil salinity on concentration of calcium, magnesium, sodium, potassium, and chloride in the plant parts.

Application of phosphorus fertilizer together with nitrogen fertilizer caused an increase of potassium concentration in leaves and roots, and a decrease of K concentration in the stems. Under the same treatment a significant decrease of calcium concentration in all plant parts was found.

Data concerning the interactive effects of nitrogen and phosphorus fertilizers with soil salinity on the concentrations of magnesium, sodium and chloride are graphically shown in Figures 5, 6, 7 and 8. From these data it could be concluded that the application of nitrogen alone, or together with phosphorus fertilizer, caused an increase of magnesium and chloride concentration and a decrease of sodium concentration in all plant parts at all soil salinity levels. Application of phosphorus fertilizer alone, or together with nitrogen fertilizer, caused a decrease of magnesium, sodium and chloride concentration in all plant parts at all soil salinity levels.

Discussion

The decrease of the dry matter weights of corn plants with the increase of soil salinity levels was a result of the direct effects of salinity which involve osmotic effect and disturbance of the nutritional balance in soil and plant (Bernestein, 1975; Bressler *et al.*, 1982; Mass and Hoffman, 1977). Data reported here confirm this hypothesis and reflect mainly the latter effect which is the disturbance of nutrition balance in soil and in the plant tissues (Hummadi, 1977).

The increase in the dry matter of the plant, and in particular the dry matter of the upper parts when nitrogen fertilizer was applied, was due to an improvement in the plant nutrition balance on the one hand, and also to the increase in the number and size of the leaves on the other hand. (Benpal, 1967; Chaargivou, 1976; Moursi, 1966). The increase in the efficiency of nitrogen fertilizer, when it was applied together with phosphorus fertilizer, in respect to the dry matter weight, was attributed to the low level of the initial content of phosphorus in

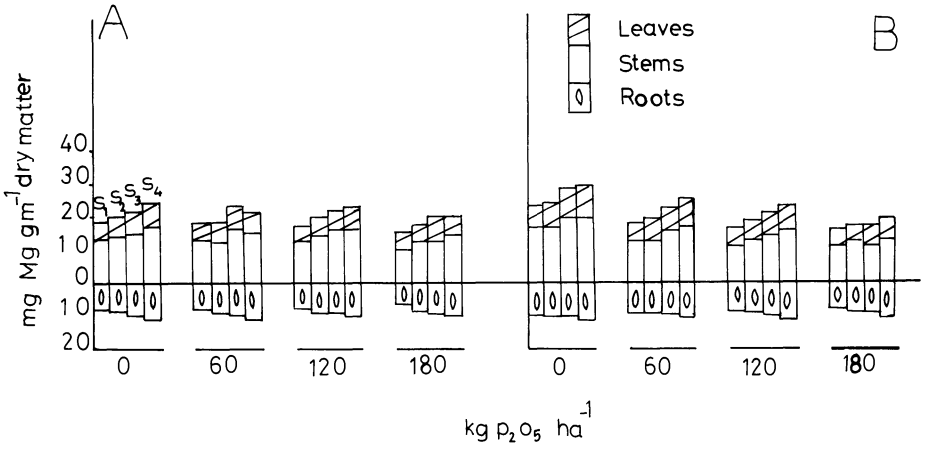

Fig. 5. Interactive effect of soil salinity and phosphorus fertilizer on magnesium concentration in the plant parts. **A**. Phosphorus and nitrogen, **B**. Phosphorus.

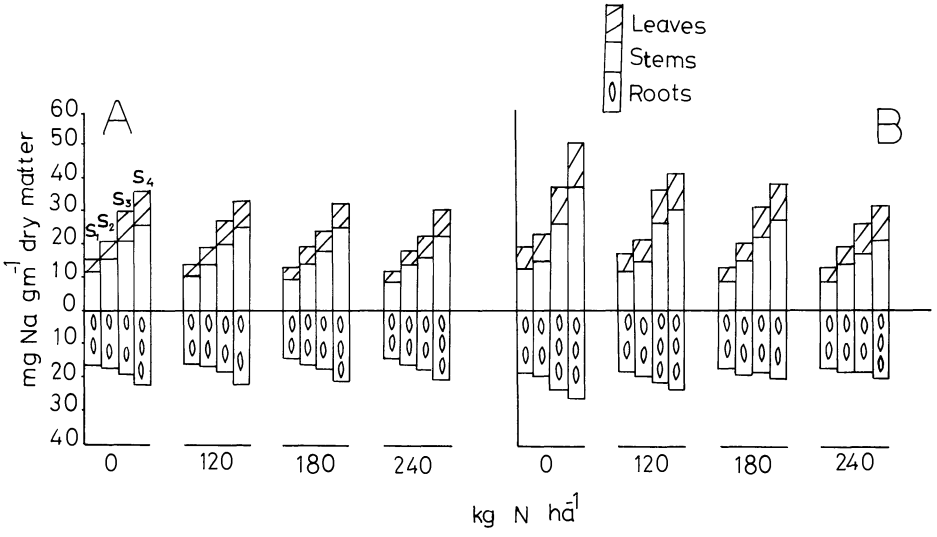

Fig. 6. Interactive effect of soil salinity and nitrogen fertilizer on sodium concentration in the plant parts. **A**. Nitrogen and phosphorus, **B**. Nitrogen.

the soil used (about 4.0 mg kg^{-1}) which is below the critical level for this soil (Hassan *et al.*, 1974). Therefore, application of phosphorus fertilizer significantly increased the efficiency of nitrogen fertilizer.

The increase of nitrogen concentration in the plant parts with increasing soil salinity was due to the decrease of the dry matter of the plant caused by increased soil salinity.

The decrease of phosphorus concentration in the plant with increasing soil salinity was attributed to the adverse effect of salinity on the roots. As a result of this effect, the surface area of the root was decreased and the absorption of phosphorus from the soil likewise decreased. The application of nitrogen fertilizer in this experiment did not increase the concentration of phosphorus in the plant because the nitrogen fertilizer primarily improved the growth of the upper part of the plant but not the roots. However, phosphorus fertilizer applied alone or together with nitrogen fertilizer caused a significant increase of phosphorus con-

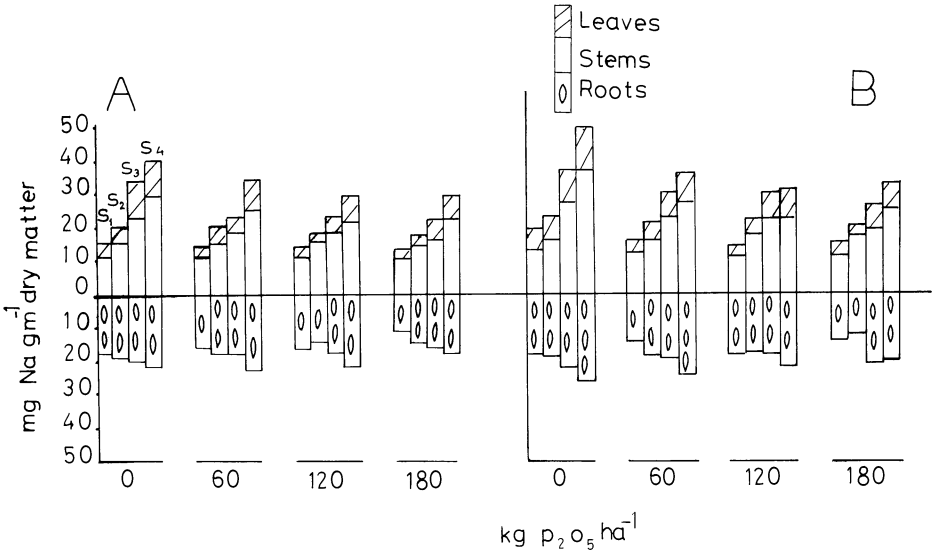

Fig. 7. Interactive effect of soil salinity and phosphorus fertilizer on sodium concentration in the plant parts. **A.** Phosphorus and nitrogen, **B.** Phosphorus.

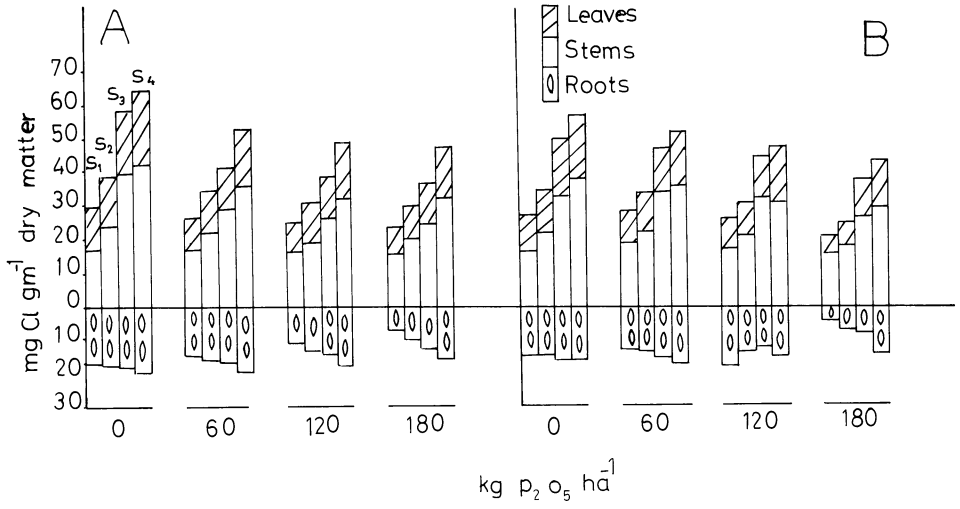

Fig. 8. Interactive effect of soil salinity and phosphorus fertilizer on chloride concentration in the plant parts. **A.** Phosphorus and nitrogen, **B.** Phosphorus.

centration. This was due to the improvement of phosphorus availability in the growth medium and assisted the roots to absorb more phosphorus.

The increase of magnesium, sodium and chloride concentration in corn with increasing soil salinity was attributed to the increase of concentration of these ions as essential salinity components in the soil with an increase of soil salinity levels. Chloride reached toxic levels (about 25.5 mg gm^{-1} of dry matter) in certain treatments such as $N_0P_0S_4$ (Teater *et al.*, 1960).

The decrease of potassium and calcium concentration in plants with increasing soil salinity was attributed to the antagonism which exists between sodium as an unavoidable presence in salinity conditions, and these two elements.

With regard to the interaction between soil salinity and nitrogen and phosphorus fertilizers in

respect of concentration of magnesium, sodium and chloride, it seems, in general, that the application of these fertilizers in doses such as used in this work, significantly improved the nutritional balance for the plant. An exceptional case should be mentioned here, *i.e.* the application of higher doses of nitrogen fertilizer under saline conditions may cause an increase of chloride concentration in plants that may reach toxic levels. Therefore, it seems, that it is not recommended to apply nitrogen fertilizer to saline soils in doses higher than those advocated for non-saline soils.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 203–208.
PLSO PN 153
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Genetic aspects of aluminum tolerance in sorghum

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Key words: acid soil, aluminum, solution culture, *Sorghum bicolor*, inheritance, heterosis, gene action

Abstract

To obtain crops tolerant to aluminum (Al) toxicity in acid soils, several methods have been used to screen different plant species and genotypes to this production constraint. Little is known about the effect of the method on genetic analyses and breeding method suggested. Three genetic studies were conducted to examine evaluation method on inheritance and gene action of sorghum [*Sorghum bicolor* (L.) Moench] to Al toxicity as measured by seedling dry matter production.

Results of acid soil and solution culture studies indicated that tolerance to Al toxicity was inherited as a dominant character. Narrow-sense heritability estimates in the greenhouse acid-soil study were low for shoot and root dry matter production. Six of the same hybrids tested in solution culture produced high additive-genetic variance and had narrow-sense heritability estimates of 72% for shoot and 65% for root DM yields. Griffing's diallel analysis showed that seven of nine restorer lines had substantially higher specific than general combining ability variances for both root and shoot dry matter yields.

Inconsistencies between the acid soil and solution culture techniques showed that different genetic responses to the treatments were being measured. The solution culture study indicated to the plant breeder that genes conditioning Al tolerance could be incorporated into pure lines while the greenhouse acid-soil study would predict that this would not be possible. The diallel study of plants grown in solution culture showed that developing both Al-tolerant varieties and hybrids would be possible depending upon Al-tolerant germplasm used. Acid-soil field studies of actual genetic gain for Al tolerance are needed.

Introduction

The term acid-soil tolerance conveys a qualitative assessment of plant adaption to acid-soil conditions under low fertilizer and lime levels (Sanchez and Salinas, 1981). The soil constraints must be characterized and crop responses to these production-limiting factors evaluated. Less than optimum conditions for plant growth are implied for conducting research of this type. Low-input systems are considered to be transitional technology in both agronomic and economic terms. They provide low-cost alternatives to shifting cultivation in highly acid soils (Sanchez and Benites, 1987).

Sorghum [*Sorghum bicolor* (L.) Moench] has been reported to be susceptible to high aluminum (Al) concentrations found in acid soils (Sanchez and Salinas, 1981). Most sorghum cultivars are not tolerant to high concentrations of Al because sorghum improvement programs have been conducted at locations with near neutral pH soils. We have previously reported a breeding program at Quilichao, Colombia in which a portion of the world collection of sorghum was screened for tolerance to tropical acid soils with a relatively high level (63%) Al saturation (Gourley, 1987a; c).

Genetic variability for Al tolerance in sorghum has been reported (Borgonovi *et al.*, 1987; Boye-Goni and Marcarian, 1985; Brown and Jones, 1977; Duncan, 1983; Gourley, 1987a; 1987b). However, relatively little information has been published on the inheritance of Al tolerance in

sorghum. Variations in Al tolerance of sorghum hybrids grown in nutrient solution from different crosses involving tolerant and susceptible parents have been found (Borgonovi *et al.*, 1987). Similar observations were noted under field conditions at high levels of Al saturation (Gourley, 1987a).

A small number of genes with dominant effects has been reported to control Al tolerance in grain sorghum (Pitta *et al.*, 1979). Evaluating F_2 and F_3 sorghum populations from tolerant-by-tolerant and tolerant-by-susceptible crosses showed that inheritance of Al tolerance in sorghum was complex and that three or more genes were involved (Bastos, 1982).

The objectives of these studies were: (1) to evaluate acid soil tolerant and susceptible sorghum parents and their hybrid combinations for tolerance to high Al concentrations and (2) to determine combining ability effects, genetic variability, heterosis, and heritability for root and shoot dry matter (DM) yield of sorghum seedlings grown in soil and nutrient solutions containing high concentrations of Al.

Table 1. Classification of 16 sorghum inbreds for aluminum tolerance and identification of country of origin

Inbred code[a]	Pedigree	Al tolerance classification[b]	Country of origin
Female (A-lines)			
1	B-Yel PI	S	USA
2	Wheatland	S	USA
3	Wheatland Der.	S	USA
Male (R-lines)			
4	IS 7542C	T	Nigeria
5	IS 12666C	S	Ethiopia
6	IS 8337C	S	Pakistan
7	3DX57/1/1/910	T	Uganda
8	NB 9040	S	USA
9	TX 430	S	USA
10	IS 2765	T	Uganda
11	IS 3071	T	Sudan
12	IS 6944	T	Sudan
13	IS 8577	T	Kenya
14	IS 8931	T	Kenya
15	IS 9084	T	Kenya
16	MN 4508	T	Uganda

[a] Inbred code number (1–16) is used hereafter instead of pedigree.
[b] S = susceptible, T = tolerant to 63% Al saturation in field screenings at Quilichao, Colombia.

Materials and methods

Genetic studies of Al tolerance in sorghum were conducted in a greenhouse using an acid-soil (Colombian oxisol at 90% Al saturation) (Experiment I) and in a laboratory using a modified Steinburg solution culture with various concentrations of Al (Experiments II and III). The 16 parental inbreds used in these studies have been evaluated for tolerance to 63% Al saturation in field trials at Quilichao, Colombia (Table 1).

Experiment I

Twelve sorghum F_1 hybrids and their parents were grown in the greenhouse at the International Center for Tropical Agriculture (CIAT), Cali, Colombia. The virgin soil used in this study was collected at Carimauga, Colombia and was an oxisol classified as a clayey, kaolinitic, isohyperthermic, Tropeptic Haplustox. Lime (to decrease Al saturation) and fertilizer were mixed with a quantity of soil to equal 2 kg and placed into plastic pots lined with black polyethylene liners.

Dolomitic lime was added to all pots at the rate of 500 kg ha^{-1}. Calcitic lime was then added to one half of the pots at the rate of 1000 kg ha^{-1}. Fertilizer and micronutrient applications consisted of 100 kg P_2O_5 and 50 kg K_2O equivalents, 100 kg urea, 10 kg borate, 7 kg copper sulfate, 7 kg zinc sulfate and 0.5 kg ammonium molybdate ha^{-1}.

Four plants per pot were grown for three weeks in a randomized complete block design with three replications. At harvest, shoots were cut at the soil surface and soil was washed from the roots. Both roots and shoots were then oven dried separately and weighed to determine DM production.

The hybrids were used to estimate genetic parameters in a North Carolina Design II analysis. This allowed estimations to be made for additive variance, dominance variance, average degree of dominance and narrow-sense heritability.

Experiment II

Six of the sorghum F_1 hybrids grown in the greenhouse genetic study were grown in solution culture in the laboratory. A modified Steinburg nutrient solution was used as previously reported

(Furlani and Clark, 1981). The concentration of P was reduced from 64 to 16 μM. Aluminum was added as $K_2Al_2(SO_4)_3 \cdot 24H_2O$ at 148 and 222 μM.

Two seedlings were suspended through holes in plastic plates over 1.9 L painted glass jars containing continuously aerated nutrient solution. Treatment jars were arranged in a randomized complete block design with three replications. Plants were allowed to grow for 21 days in the solution. The pH of the solution was monitored and adjusted to pH 4 with 1 M HCl as needed. Light was provided by white fluorescent bulbs located 90 cm above the bench. The daily light regime was 17 hours of light and 7 hours of darkness. The temperature in the laboratory was 20–25°C.

Harvested shoots and roots were oven dried and weighed. Data from the six hybrids were analyzed as in Experiment I.

Experiment III

Nine sorghum restorer lines (R-lines) were selected for this study. A diallel cross, without reciprocals, among the nine parental inbreds was made by hand emasculation. The equipment and evaluation procedure was the same as in Experiment II except the plants were grown for a total of 28 days before harvest.

The diallel cross (36 hybrids) was evaluated for Al tolerance at two concentrations of Al, 77 μM (control) and 222 μM (stress level). Only data collected from the hybrids grown at 222 μM Al were used for genetic analyses.

The experimental design was a randomized complete block with four replications. The diallel analysis developed by Griffing (model 1, method 4) was used for the analysis of DM yield of the total plants.

Results and discussion

Experiment I

There were significant differences for all variables between lime rates and among genotypes. Significant lime × genotype interactions were found for shoot DM yield only. When shoot and root DM was compared in the 500 kg ha^{-1} lime treatment, few of the hybrids produced more DM than either of their parents (Table 2). Several hybrids produced more DM than one parent and were equal to the other parent. At 1500 kg ha^{-1} lime, a majority of the hybrids produced larger shoot and root DM yields than either of their parents.

Table 2. Evaluation of 12 sorghum F_1 hybrids and parents grown in acid soil at two lime rates in the greenhouse

Genotype	500 kg lime ha^{-1} (80% Al saturation)		1500 kg lime ha^{-1} (71% Al saturation)	
	Shoot DM[b]	Root DM[b]	Shoot DM	Root DM
P1 × P4	102 a[a]	140 a	167 ab	140 a
P2 × P4	91 a–c	96 a–c	133 bc	109 a
P3 × P4	83 ab	100 a–c	140 bc	114 a
P1 × P5	92 a–c	115 ab	183 a	126 a
P2 × P5	43 d–f	55 c	80 d–f	64 b
P3 × P5	95 ab	86 bc	130 bc	121 a
P1 × P6	85 ab	100 a–c	138 ab	119 a
P2 × P6	83 a–d	88 bc	97 c–e	105 a
P3 × P6	81 a–e	72 bc	121 c	125 a
P1 × P7	89 a–c	76 bc	106 cd	56 b
P2 × P7	86 a–d	83 bc	127 bc	110 a
P3 × P7	100 a	80 bc	110 cd	68 b
P1	41 d–f	68 bc	53 f	61 b
P2	51 b–f	54 c	58 ef	53 b
P3	42 d–f	51 c	46 f	44 b
P4	58 b–f	41 c	76 d–f	58 b
P5	39 ef	55 c	60 ef	48 b
P6	32 f	39 c	52 f	49 b
P7	47 c–f	49 c	95 c–e	47 b

[a] Means within a column followed by a common letter are not significantly different at the 0.05 level of probability according to Student–Newman–Keuls' test.
[b] Shoot and root dry matter in mg plant^{-1}.

Genetic components of Al tolerance were estimated for shoot and root DM production at each lime level (Table 3). Genetic estimates at both lime rates showed high dominance variance and average degree of dominance which indicated that a large portion of the Al tolerance was due to non-additive gene action. The narrow-sense heritability estimates were a reflection of the additive portion of the genetic variance and were consequently low. This study showed that Al-tolerance genes, as indicated by the variables measured using this evaluation technique, could not be utilized in variety improvement in a breeding program.

Experiment II

There were significant differences among

Table 3. Genetic components of 12 sorghum F_1 hybrids grown in acid soil at two lime rates and six F_1 hybrids grown in solution culture containing 222 μM Al

Component	Soil study (Expt. I)				Solution culture (Expt. II)	
	Shoots		Roots			
	500	1500	500	1500	Shoots	Roots
Additive variance	576	7348	7360	4008	4896	5084
Dominance variance	6321	33628	9664	30200	0	1660
Ave. degree of dominance	5.4	3.0	1.6	3.9	0.0	0.6
Heritability (%)	5	16	31	8	72	65

genotypes and between Al concentrations. The only significant Al × genotype interaction was for shoot DM production.

The F_1 hybrids tended to exceed one or both parents for DM production (Table 4). However, data for all male parents were not available for this study (P4 was lost) which limited comparisons between hybrids and parents. It appeared that 148 μM Al was probably not adequate for separating tolerant and sensitive sorghum genotypes. Therefore, the genetic components for Al tolerance, as measured by DM production, were estimated in this study only for the 222 μM Al treatment.

In solution culture, additive variance was much greater than dominance variance (Table 3). Shoot DM yields indicated no dominance, while root DM yields indicated partial dominance. Narrow-sense heritability estimates for each component were high, indicating additive gene action which can be concentrated in a homozygous state in a breeding program.

Table 4. Evaluation of sorghum hybrids and inbreds at two concentrations of Al in nutrient solution

Genotype[a]	148 μM Al		222 μM Al	
	Shoot DM[c]	Root DM	Shoot DM	Root DM
P1 × P4	121 ab[b]	108 a	88 ab	87 a
P2 × P4	76 b–d	81 a–c	83 a–c	75 ab
P3 × P4	84 b–d	72 a–d	58 a–f	50 a–d
P1 × P6	68 cd	57 b–d	43 b–f	30 b–d
P2 × P6	90 b–d	61 a–d	59 a–f	38 a–d
P3 × P6	39 d	44 cd	33 c–f	32 b–d
P1	58 cd	42 cd	33 c–f	16 d
P2	50 d	28 d	69 a–f	49 a–d
P3	52 d	39 cd	43 b–f	28 b–d
P6	38 d	37 cd	17 f	13 d

[a] Inbred P4 was lost in this study.
[b] Means within a column followed by a common letter are not significantly different at the 0.05 level of probability according to Student–Newman–Keuls' test.
[c] Shoot and root dry matter in mg plant^{-1}.

Experiment III

Results presented in Table 5 showed that 222 μM Al significantly reduced DM yield of roots and shoots of the parental genotypes compared to the control. Three of these inbreds (P10, P12, and P14) produced relative root and shoot DM yields greater than 80% of the control.

Treatment mean squares for hybrids, general (GCA) and specific combining ability (SCA) from Griffing's diallel analysis for the three measured yield variables of the nine-parent diallel grown in solution containing 222 μM Al were all significant

Table 5. Mean root and shoot dry matter yields of nine 4-week-old sorghum parental genotypes grown in solution culture containing 77 and 222 μM Al

Genotypes	Root weight (mg)		RRW[a] (%)	Shoot weight (mg)		RSW[a] (%)
	77 μM	222 μM		77 μM	222 μM	
P8	58 e[b]	26 d	46	74 e	42 d	56
P9	71 e	17 d	24	104 de	24 d	23
P10	236 bc	209 a	88	256 bc	231 a	90
P11	270 b	146 b	54	328 b	170 ab	52
P12	140 d	134 b	96	168 cd	151 bc	89
P13	207 bcd	146 b	70	241 c	180 ab	74
P14	155 d	130 b	84	200 c	166 b	83
P15	372 a	128 b	34	444 a	172 ab	39
P16	182 cd	90 c	49	212 c	105 c	50

[a] RRW = relative root weight, RSW = relative shoot weight and 222/77 μM Al (as percentage).
[b] Means in a column not followed by the same letter are significantly different at the 0.01 level of probability according to the Student–Newman–Keuls' test.

Table 6. Estimates of general and specific combining ability effects for dry matter yield of total plants from a nine-parent diallel grown in solution culture containing 222 μM Al

Genotypes	GCA effects	SCA effects							
		P9	P10	P11	P12	P13	P14	P15	P16
P8	−51**	−153**	−7	−19	−29	−82**	−95**	242**	143**
P9	−102**		148**	−10	104**	−30	−118**	−46	107**
P10	147**			−140**	140**	41	−28	−201**	48
P11	−13				−55*	220**	226**	−54*	−168**
P12	14					−136**	−80**	153**	−98**
P13	5						52	−64*	0
P14	55*							22	20
P15	−42*								−51
P16	−12								—

*, ** Significant at the 0.05 and 0.01 level of probability, respectively according to the t test.

($P < 0.01$). Estimates of GCA and SCA effects for DM yield of roots and shoots were generally similar, therefore only effects for total plant DM yield are shown in Table 6. Inbreds P10 and P9 had the highest and lowest GCA effects, respectively. Of the Al-tolerant parents, only P10 and P14 had significantly positive GCA effects. Hybrid P8 × P15 produced the highest SCA effect (241.5).

Partitioning the genetic variance associated with each parent into GCA and SCA variances for total plant yield showed that P10 uniformly transmitted its high yielding ability to most of its F_1's as indicated by its high GCA variance (Table 7). The other good general combiner, P14, attained its high average performance primarily by a different means. The relatively high SCA variance associated with this inbred indicated that specific combinations of P14 with certain other inbreds yielded considerably more than would be expected and other combinations which yielded much less than expected. Although the other inbreds were poor general combiners, they generally attained their average hybrid performance by means of their relatively higher SCA variance.

Table 7. Estimates of general and specific combining ability variances associated with each parent for dry matter yield of the total plants for a nine-parent diallel grown in solution culture containing 222 μM Al

Genotypes	Vgi[a]	Vsi[b]
P8	2134	1371
P9	9968	8775
P10	20984	11883
P11	−321	18699
P12	−280	9876
P13	−466	8609
P14	2527	8784
P15	1279	15926
P16	−340	7445

[a] Vgi = general combining ability variances. Negative variance values are considered to be zero.
[b] Vsi = specific combining ability variances.

Summary and conclusions

Inconsistencies between greenhouse soil and solution-culture experiments, using some hybrids in common, showed that different genetic responses to the treatments were being measured. The solution-culture study indicates to the plant breeder that genes conditioning Al tolerance could be incorporated into pure lines while the greenhouse soil study predicts that this would not be possible. Both studies do show the advantage of hybrid vigor for Al tolerance.

The diallel evaluated in solution culture indicated that Al tolerance in sorghum was dominant to Al susceptibility and involved both additive and non-additive gene action. The additive portion of the genetic variance could be fixed through recombination and selection in a breeding program. The large SCA variances associated with several of the selected inbreds could best be utilized in hybrid combinations.

The three experiments demonstrate that Al tolerance in sorghum involves both additive and non-additive gene action. The type of gene action controlling Al tolerance appears to be partially dependent upon the degree of Al stress, technique employed, and germplasm being evaluated.

Acknowledgement

This research was sponsored by the USAID funded International Sorghum/Millet (INTSORMIL) Collaborative Research Support Program Grant No. AID/DAN-1254-G-SS-5065-00. The authors thank CIAT for the use of facilities. Journal contribution No. J 6945 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762.

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PLSO PN 154

Influence of salt stress on primary metabolism of *Zea mays* L. seedlings of model genotypes

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Key words: growth chamber, nutrient solution, $^{15}NO_3$ assimilation, photosynthesis, relative growth rate, seedlings, short-term NaCl stress

Abstract

Short-term studies for comparing some primary metabolic and growth-responses to salt stress in seedlings of two maize genotypes differing in drought resistance were carried out under controlled conditions. Both genotypes revealed high yielding ability in favourable environments. Treatments: Control (Hoagland-Arnon No 1 solution) and salt stress (Hoagland-Arnon solution plus NaCl, $\psi_s = -0.84$ MPa). It was found that in both genotypes the activity of the principal metabolic pathway supplying reduced nitrogen (^{15}N) for the synthesis of amino acids and proteins as well as the assimulatory number ($^{14}CO_2$ — assimilation relation rate per chlorophyll unit) were decreased under the effect of the stress. These effects were more marked in the resistant genotype. In this genotype the stress induced metabolic activity decline was accompanied by a corresponding reduction of the relative growth rate. Conversely, continuing growth, resulting probably from accumulation of solutes, was observed in the susceptible genotype.

On the basis of these and other observations it is assumed that the resistant genotype manifests short-term energy saving stress reactions.

Introduction

The comparative study of genotypes resistant and susceptible to stress is a possible approach for studying the adaptive significance of the stress-induced metabolic changes (Hanson and Hitz, 1982). Here we present part of the studies carried out with two genotypes of maize, whose 'biography' has been checked in advance with respect to the parameters of grain yield and resistance to drought. The genotypes have high yields under favourable field conditions, one of them being susceptible to drought and the other one drought resistant. The aim of the present work was to analyse the effect of a short-term salt (NaCl) stress on nitrate reductase and on nitrate assimilating activities, as well as on the CO_2 assimilation intensity, under controlled conditions.

Materials and methods

Genotypes investigated: 9-day-old seedlings of two F_1 single-crosses (Genotype H_1 — drought resistant and Genotype H_2 — drought susceptible).

Seeds prepared for germination were surface sterilized with $Ca(OCl)_2 \cdot 3\ H_2O$ for 20 min., followed by soaking for 1 h in running water (18°C). Filter paper rolls, each containing 10 seeds were placed vertically in 250 mL beakers with 100 mL distilled water, and maintained in complete darkness throughout the period of germination (72 h). When coleoptiles were 2 — 2.5 cm long, the seedlings were transferred to an environmental chamber with day-night air temperature 23 — 18°C, 12 h photo-period (12.000 lx), and 75% r.h. Hoagland-Arnon No 1 solution was used (5 m*M* NO_3 ions).

Treatments

Control: Hoagland-Arnon solution. Salt-stress treatment: Hoagland-Arnon solution plus 170 m*M* NaCl ($\psi_{s\,ext.} = -0.84$ MPa). When PEG treatment was involved in the investigations to compare the osmotic stress produced by both water and salt stresses, PEG_{6000} was used as a nonpenetrating osmoticum, producing changes similar to those caused by drying soil at the same water potential (Heyser and Nabors, 1981). Isosmotic solutions of PEG and NaCl were prepared using the same nutrient solution. PEG was isosmotic to 170 m*M* NaCl.

Stress exposure was for 24 h before starting the analyses

In vitro assay for NADH-Nitrate reductase activity (NRA, EC 1.6.6.1.) was carried out as described elsewhere (Voronova *et al.*, 1976) using a standardized extraction medium (Mladenova *et al.*, 1982). Standard deviation was in the range ± 0.5 *i.e.* 5.4%.

^{15}N analyses

44 h before harvesting, the seedlings were exposed to nutrient solution containing $^{15}NO_3$ (50 atm % enrichment with ^{15}N). Sample preparation for isotope-ratio analyses involved three conventional steps (see Hauck and Bremner, 1976). Emission spectrometry was performed using a Statron Model NOI-3. Standard dev. was ± 0.5 *i.e.* 2%.

Photosynthetic $^{14}CO_2$ assimilation rate and plastid pigment content determinations as well as delayed fluorescence measurements were made as previously described (see Stoilov *et al.*, 1985, Goltsev *et al.*, 1984).

Results

A stress-induced decrease of the rate of nitrate reduction was observed in both genotypes, as well as a reduction of the nitrate assimilating activity, which was assessed by the ^{15}N-enrichment of the total reduced nitrogen after 44 h exposure with $^{15}NO_3$ ions in the nutrient medium (Table 1). The decrease of the *in vitro* nitrate reductase activity (NRA) and of the rate of NO_3 assimilation was more pronounced in the drought-resistant genotype H_1 than in the drought-susceptible genotype H_2 (Fig. 1). It is seen from Table 1 and Fig. 1 that the intensity of the integral photosynthetic process remained practically unchanged for both genotypes as a result of the salt

Table 1. Changes of nitrate assimilating and photosynthetic activities of growth-chamber grown seedlings of genotype H_1 and genotype H_2 in response to short-term (24 h) salt stress (NaCl, ψ_s ext. $= -0.84$ MPa)

Parameter	Treatment	Genotype H_1 (drought resistant)		Genotype H_2 (drought susceptible)	
		Shoots	Roots	Shoots	Roots
^{15}N atom % excess[a]	Control	0.95	1.96	1.36	3.16
	NaCl	0.62	1.56	0.94	3.39
Total reduced	Control	23.75	19.99	33.32	48.66
^{15}N (μg) per seedling	NaCl	15.62	14.98	23.31	44.07
In vitro NADH-NRA	Control	72.9		72.5	
(EC 1.6.6.1.)	NaCl	51.9		60.5	
n*M* NO_2^- min^{-1} g^{-1} fr. wt					
Rate of Photosynthesis	Control	13.4		16.2	
(mg $^{14}CO_2$ dm^{-2} h^{-1})	NaCl	12.9		15.6	
Assimilatory number	Control	8.24		7.63	
(Rate of photosynthesis	NaCl	6.32		7.89	
per chlorophyll unit)					
$^{15}NO_3^-$-assimilation rate	Control	58.49		81.78	
(Total red. ^{15}N per plant/	NaCl	44.05		72.27	
D.M. roots per plant)					

[a] Exposure to ^{15}N-NO_3^- in No 1 nutrient solution of Hoagland-Arnon—44 h.

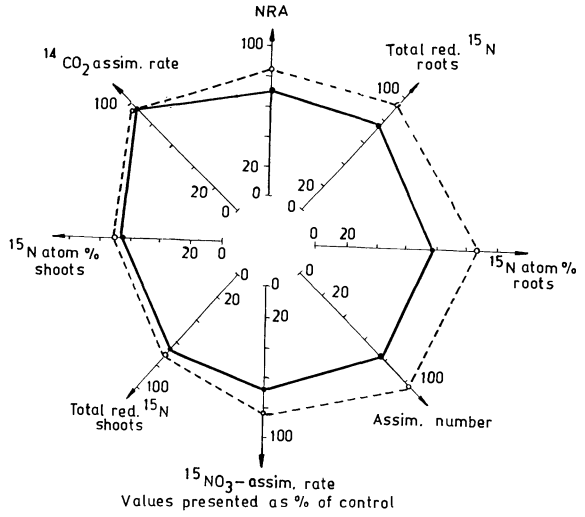

Fig. 1. Polygonal diagram for comparing the salt induced responses (relative to the respective nonstressed control) of genotypes H_1 (——) and H_2 (- - - -). *Axes of the diagram* represent the same parameters as shown in Table 1.

stress imposed. However, the assimilatory number, which is the efficiency of chlorophyll for photosynthetic assimilation of $^{14}CO_2$, decreased by 23 per cent in genotype H_1 in response to the salt stress, but remained unchanged for genotype H_2 (Fig. 1).

These results showed the probable occurrence of specific changes in the plastid pigment complex of the genotypes. Indeed, it was found later that in genotype H_1 the salt stress caused a substantial rise (in % to the untreated control) of the content of chlorophyll *a* (25%), of chlorophyll *b* (28%), and of carotenoids (28%)—the data are shown in Fig. 2. It can be seen that such changes were absent in genotype H_2. A parallel study with the application of PEG-6000 showed that the changes observed in genotype H_1 are not due to osmotic stress, being a specific response of this genotype to the ionic effects caused by the increased NaCl concentration.

The data shown on Fig. 2 suggests that salt stress may also cause other differential genotypic changes in the photosynthetic apparatus. Subsequent studies confirmed this assumption. Specific genotypic changes were observed in the induction kinetics of the millisecond delayed fluorescence of the chlorophyll *in vivo*, in response to the experimented salt stress (Goltsev and Mladenova, 1987, Mladenova *et al.*, 1987). In genotype H_2 essential differences were found between the PEG- and NaCl-induced changes in the light curves of the milli-second delayed chlorophyll fluorescence. These differences were best manifested in the zone of the half-saturating intensity of exciting light I_{50} (Fig. 3). It can be seen from the Figure that no such differences occurred in genotype H_1.

In genotype H_1 it was found that the growth response to salt stress decreased parallel to the drop of the activity of the reactions monitored (Fig. 4).

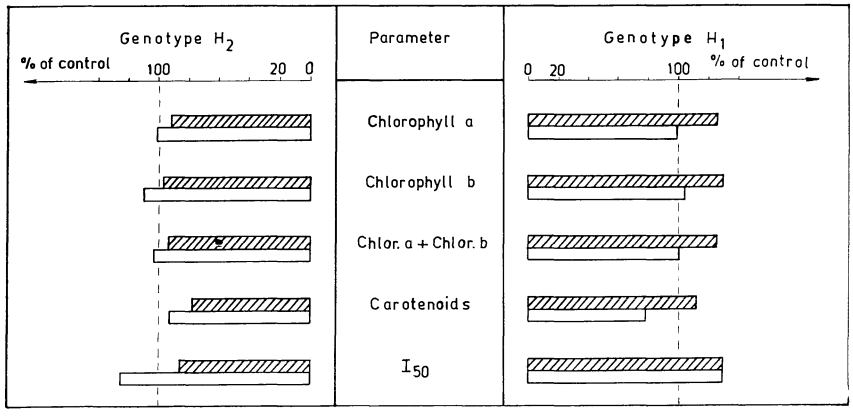

Fig. 2. NaCl (▨) and PEG (□) induced changes in plastid pigment content and I_{50} of growth-chamber grown seedlings of two maize genotypes differing in drought tolerance. Genotype H_1 is drought resistant and genotype H_2—drought susceptible. PEG is isoosmotic to 170 m*M* NaCl (ψ_s ext. = −0.84 MPa) I_{50} = half saturating intensity of exciting light, being a representative characteristic of light curve of milli-second delayed chlorophyll fluorescence (see Fig. 3).

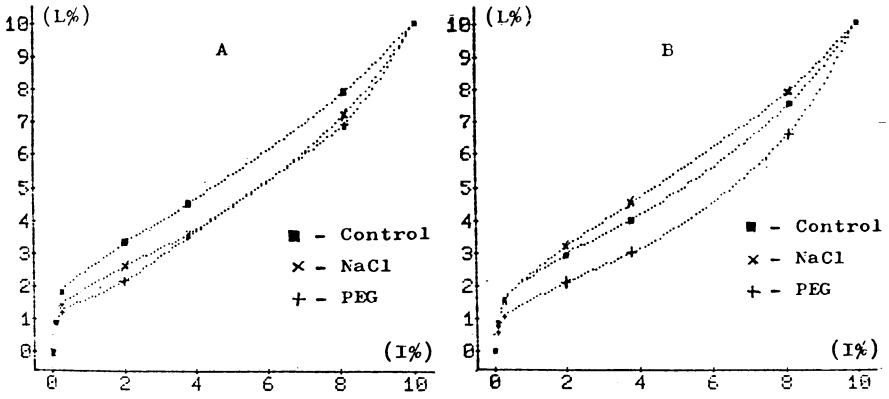

Fig. 3. Salt and water stress responses of light curves of milli-second delayed fluorescence (DF) of intact leaves of two *Zea mays L.* genotypes at the seedling stage. I = intensity of exciting illumination (% of maximal intensity of exciting light). L = Intensity of DF (% of DF at maximal intensity of exciting light). I_{50} = Half saturating intensity of exciting light. **A:** Responses of genotype H_1 (drought resistant). I_{50}(PEG) = 128.1% of control. I_{50}(NaCl) = 128.8% of control. **B:** Responses of genotype H_2 (drought susceptible). I_{50}(PEG) = 125.5% of control. I_{50}(NaCl) = 84.9% of control. (Adapted from Mladenova *et al.*, 1987.)

Conversely, in genotype H_2 the relative growth rate increased, in spite of the salt-induced decrease of the nitrate assimilating activity. It can also be seen from the figure that in genotype H_1 there were no specific ionic effects of the salt stress on growth, which is demonstrated by the similarity of the PEG- and NaCl-induced changes in the relative growth rate of the shoots and roots of this genotype. Conversely, a difference in the responses of the relative growth rate was observed in genotype H_2, depending on whether the stress was applied through PEG or through NaCl (Fig. 4).

The water content in the leaves and roots of both genotypes was found to remain practically unchanged as a result of water (PEG) and salt (NaCl) stresses (Table 2). It can also be seen from the table that while PEG- and NaCl-induced changes in the fresh weight of the shoots were similar, there was a difference in the NaCl- and PEG-induced responses of the root fresh weight,

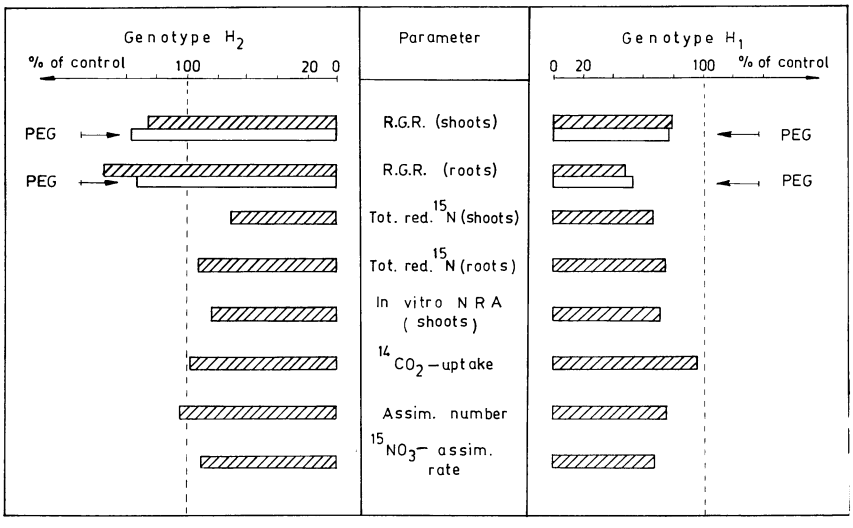

Fig. 4. Summated data showing the differential genotype stress induced growth changes and the corresponding salt stress responses of nitrate assimilation and photosynthetic activities of the resistant genotype H_1 and of the susceptible genotype H_2.

Table 2. Short-term (24 h) salt-(NaCl) and water (PEG)-induced responses of water content (W.C.) and fresh weight (fr. wt) of 9-day-old seedlings of genotype H_1 and genotype H_2 under growth-chamber conditions. (PEG_{6000} is isoosmotic to 170 m*M* NaCl ψ_s ext. = −0.84 MPa)

Parameter	Treatment	Genotype H_1 (drought resistant)		Genotype H_2 (drought susceptible)	
		Shoots	Roots	Shoots	Roots
W.C.	Control	92.4	90.1	90.3	89.8
(% of fr. wt)	NaCl	92.2	90.6	90.5	90.0
	PEG	92.0	90.1	89.9	89.5
fr. wt	Control	100.0	100.0	100.0	100.0
(% of	NaCl	92.5	96.9	105.4	116.0
control)	PEG	89.8	92.6	100.0	102.2

especially in genotype H_2. This shows that the change was not related only to a water deficit developing under salt stress conditions.

Discussion

The observed salt-induced decrease of the metabolic activity of genotype H_1, accompanied by the respective growth decrease (Fig. 4) could be discussed in the light of the assumption that such a decrease would make the plants less sensitive to the stress effects (Gershenzon, 1984). This assumption is in agreement with other data, which suggest that a reduction in growth under saline conditions, accompanied with salt exclusion in some crops, could be a mechanism of coping with such conditions (Läuchli, 1984). Maize is found to possess the capability of excluding sodium in such an environment (Draw and Läuchli, 1985), and also a considerable variability is revealed among maize cultivars with respect to this capability (Maas *et al.*, 1983). Therefore, it is possible that the decrease in growth observed in genotype H_1 is a genetically determined adaptive response to the short-term increased concentration of NaCl in the root medium, *i.e.* that it is perhaps an energy-saving stress reaction. Incidentally, such is the reaction also of the stress tolerant wild species (see Tal, 1985).

However, cases are reported in which no differences between the genotypes were established with respect to the dry matter, in spite of differences in the capability for salt exclusion (see Schubert and Läuchli, 1986).

The observed continuing intensive growth of genotype H_2 in response to short-term NaCl stress could be linked to the following circumstances: (a) maize is classified as a moderately salt-sensitive crop, but the intraspecific variation with respect to the growth responses induced by salt stress in this crop, as well as in most glycophytic crops (with the exception of wheat, barley and rice) has not been investigated on a wide genotypic basis (Downton, 1984); (b) It may be expected that there are individual genotypes within one non-halophytic species, which manifest halophytic salt-stress responses, and vice versa (Downton, 1984; Gorham *et al.*, 1985); (c) The leaves of 9-day-old maize seedlings are expanding leaves with monocotyledons-specific growth characteristics. We are referring to a possibility for osmotic adjustment with accumulation of solutes under conditions of osmotic shock, a reaction which is absent in the expanding leaves of the dicotyledons (Barlow, 1986).

However, what could be the long-term effects of the halophytic response observed in genotype H_2? As we have seen in Figure 4, the nitrate assimilation was reduced in both genotypes as a result of the salt stress. Consequently, the growth in genotype H_2 cannot be expected to last long without guaranteeing the synthesis of molecules needed for the cell growth, which also depends on the supply of reduced nitrogen. It is possible that the cell increase in genotype H_2 depends temporarily more on the extensibility of the cell wall (see Cosgrove, 1983), providing a certain additional volume for the accumulation of solutes, than on the synthesis of new molecules needed for the cell wall growth. It

should be pointed out that the accumulation of solutes is an energy-consuming osmotic work.

A great genotypic variation is known to exist both with respect to the elastic properties of the cell wall (see Turner, 1986) and with respect to the nature of the accumulating solutes (Aspinall, 1986; Hanson and Hitz, 1982; Wyn Jones, 1984) under conditions of water and salt stresses. However, there are many unresolved problems connected with these studies (Aspinall, 1986; Gorham *et al.*, 1985; Turner, 1986).

It is assumed that leaf expansion of non-halophytes as *e.g.* maize, at short-term high salinity is regulated by a message from the roots (Munns and Termaat, 1986).

As we have seen, the short-term salt stress applied in both genotypes practically did not change the intensity of the integral photosynthetic process (Table 1), which does not mean, however, that no specific ionic effects have been exercised on some properties of photosynthetic apparatus of the two genotypes (see Fig. 2 and Fig. 3). As can be seen from Figure 3, the salt stress induced a decrease of I_{50} of the order of 15% compared with the control, which could be assumed to be equivalent to a delayed rate of the electron transport between the two photosystems (Venediktov *et al.*, 1982). Such a reaction is absent in genotype H_1, where an increase in I_{50} of the order of 28% was observed both for the water and for the salt stress. The observed responses in genotype H_1 which were common for the different stresses applied may be connected with a genetically conditioned capacity of this resistant genotype for unifying some stress reactions. It is known at the same time that each unification is considered usually as being a sign for an increased efficiency.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 215–219.
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PLSO PN 155

Genetic control of aluminium tolerance in sorghum

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Key words: aluminium, inheritance, root growth, *Sorghum bicolor* (L.) Moench

Abstract

A half-diallel cross involving two Al-tolerant (IS7173 and IS12666) and two Al-sensitive (Tx415 and IS1143) sorghum genotypes and their F_2 generations was used to study the inheritance of Al tolerance in grain sorghum. Ten-day-old plants from the parents, F_1, and F_2 populations from the crosses were grown for fifteen days in Al toxic nutrient solutions (167 μM Al) and the longest adventitions root length (LARL) was used as a plant trait for aluminium tolerance evaluation. Al tolerance was a dominant trait since F_1 generation from the crosses involving Al-tolerant and Al-sensitive genotypes yielded plants as tolerant as the Al-tolerant parent. Plants from the F_2 generations segregated into two classes and the ratio was from 3 tolerant to 1 sensitive, typical of a single gene inheritance. Broad sense heritability was high which indicates that plant selections in F_2 generations should be effective and might help the breeding programs to adapt sorghum for Al-stressed soils.

Introduction

The reclamation of many soils having high acidity and aluminium (Al) toxic subsoils is not always economically feasible by usual liming practices. In this situation the use of Al-tolerant germplasm becomes an attractive approach to better exploit these problem soils. Genotypic variability for Al tolerance in sorghum has been shown from both Al-toxic soils and nutrient solutions experiments (Borgonovi *et al.*, 1987; Duncan, 1981; Duncan *et al.*, 1983; Furlani and Clark, 1981; 1987; Furlani *et al.*, 1987; Gourley, 1987; Konzak *et al.*, 1976; Malavolta *et al.*, 1981; Salinas and Sanches, 1979; Schaffert *et al.*, 1975). However, only a few reports are available about the gene action mechanism for Al tolerance in sorghum. Some preliminary data have indicated that a few genes with dominant effect are involved in controlling Al tolerance in sorghum (Pitta *et al.*, 1979). More recently, Bastos (1982) suggested that three or more genes are responsible for the Al tolerance mechanism. Boye-Goni and Marcarian reported that the degree of Al tolerance was dominant and due to additive gene effects. Borgonovi *et al.* (1987) concluded that the inheritance of Al tolerance in sorghum appeared to be complex and that there are few major genes with a dominant trend and several minor genes with, at least, additive effects involved in the genetic control for tolerance to Al in sorghum. The objective of the present paper was to study the type of genetic control involved in the Al tolerance expression by six segregation populations derived from a half-diallel cross among two-tolerant and two Al-sensitive sorghum genotypes.

Materials and methods

A four-parent half-diallel cross involving two Al-tolerant IS7173 (SC283) and IS12666 (SC175.14) and two Al-sensitive IS1143 (SC208) and Tx415 (7078 derivative) sorghum lines were made by hand emasculation. In crosses between Al-sensitive (Tx415 or IS1143) and Al-tolerant (IS7173 or IS12666), the Al-sensitive genotype was used as the female to test each F_1 plant to certify that no selfcrossing of the Al-sensitive female parent was made. This was done by growing F_1 plants in Al-toxic nutrient solutions (4.5 mg of

Table 1. Population size, mean, variance, range and genetic estimates in six crosses of sorghum

Cross no	Population	n	LARL	Variance	Range	Potency ratio	Tolerant: Susceptible Ratio		Probability[a]	Broad sense heritability
							Expected	Observed		
1	Tx415 (P_1)	36	2.06	0.3600	1.1–3.2	—				
	IS1143 (P_2)	36	1.86	0.0900	1.3–2.4					
	$P_1 \times P_2$ (F_1)	67	3.05	0.4096	1.8–4.6					
	$P_1 \times P_2$ (F_2)	252	2.25	0.3844	1.1–4.5		0:252	0:252	—	0.26
2	Tx415 (P_1)	36	3.32	0.7225	1.3–4.2	+0.9				
	IS12666 (P_3)	36	26.12	18.9912	15.7–35.0					
	$P_1 \times P_3$ (F_1)	67	24.80	21.6843	13.4–32.3					
	$P_1 \times P_3$ (F_2)	252	14.50	71.2891	1.1–31.0		189:63	189:69	0.30–0.50	0.69
3	Tx415 (P_1)	35	2.41	0.7396	1.1–3.8	+0.4				
	IS7173 (P_4)	36	31.84	32.0356	22.1–40.7					
	$P_1 \times P_4$ (F_1)	54	22.75	17.6400	15.6–31.2					
	$P_1 \times P_4$ (F_2)	252	17.81	119.9025	1.6–43.5		189:63	196:56	0.30–0.50	0.86
4	IS1143 (P_2)	36	1.42	0.0400	1.0–1.7	0.0				
	IS12666 (P_3)	36	24.75	6.0025	18.0–28.7					
	$P_2 \times P_3$ (F_1)	34	12.96	7.1289	7.9–18.2					
	$P_2 \times P_3$ (F_2)	252	10.80	41.0881	1.3–23.7		189:63	186:66	0.80–0.90	0.89
5	IS1143 (P_2)	36	1.99	0.4225	1.2–3.7	+0.9				
	IS7173 (P_4)	36	33.74	24.6016	21.0–44.0					
	$P_2 \times P_4$ (F_1)	70	32.11	29.9209	17.3–42.0					
	$P_2 \times P_4$ (F_2)	252	22.99	161.0361	1.3–46.6		189:63	196:56	0.30–0.50	0.88
6	IS12666 (P_3)	35	25.12	27.0400	17.6–39.4	—				
	IS7173 (P_4)	36	31.51	29.1600	18.5–41.9					
	$P_3 \times P_4$ (F_1)	72	32.29	50.1264	17.2–47.8					
	$P_3 \times P_4$ (F_2)	252	33.56	76.7376	11.7–52.2		252:0	252:0	—	0.54

[a] Probability that the data is in conformity with a 3:1 ratio, according to Chi-Square test.

$Al l^{-1}$). F_1 plants that behaved like the female plants were discharged; the remaining F_1 plants were transferred to a fertile soil and allowed to grow until seed physiological maturity and then the panicles were harvested and threshed. Special care was taken to cover the panicles before flowering to ensure selfing of the F_1 and yield of pure F_1 seeds. Seeds from the four parents, F_1 and F_2 populations were germinated between moist rolled paper towels. Ten-day-old seedlings were transferred to plastic plates that held up to 66 plants (wrapped loosely with sponge rubber) and grown for 15 days in 15 liters of 0.5-strength nutrient solution containing 167 μM Al. At the termination of the experiments, the length of the longest adventitious root (LARL) was measured on individual plants. For cross No. 4, plants from parents, F_1 and F_2 generations were also grown in 0.5-strength nutrient solution with no added Al. Nutrient solution composition and handling of plants have been described elsewhere (Furlani and Clark, 1981; 1987). After measurement, plants from F_2 generations from each cross were classified as tolerant (LARL > 5.0 cm) or susceptible (LARL < 5.0 cm) and the data were analysed according to Stansfield (1969). The potency ratio, an estimate of dominance, was calculated by dividing the deviation of the F_1 from the mid parent by the deviation of the high parent from the mid parent (Petr and Frey, 1966). Broad sense heritabilities were equal to the difference between the F_2 variance and the square root of the product of the parental variances divided by the F_2 variance (Petr and Frey, 1966).

Results and discussion

The parental lines, F_1 and F_2 families from the

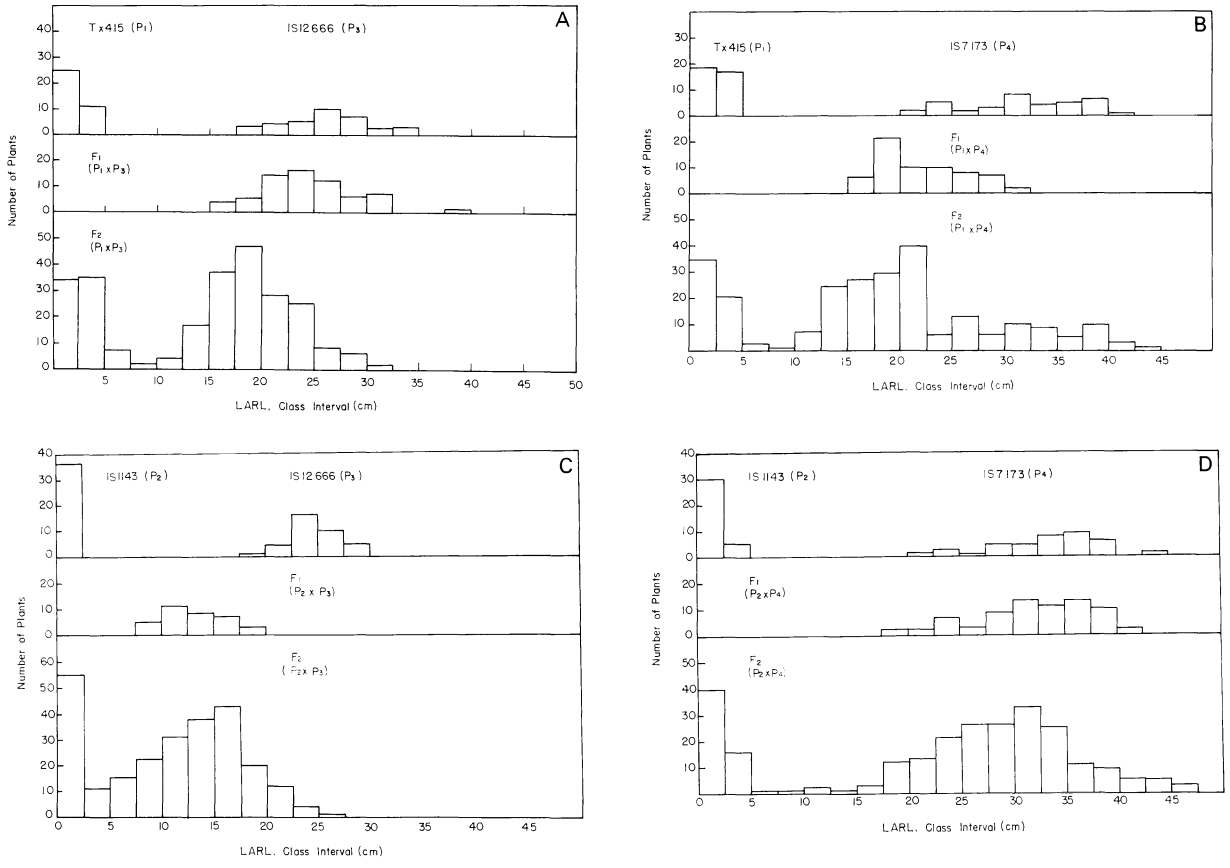

Fig. 1. Frequency distribution for LARL values in parents, F_1, and F_2 generations from the crosses: **A.** T × 415/IS12666, **B.** Tx415/IS7173, **C.** IS1143/IS12666, and **D.** IS1143/IS7173.

cross IS1143/IS12666 grown in nutrient solutions with no added Al, did not show any differences in root growth. The mean values were: IS1143 = 25.63 ± 2.57 IS12666 = 24.58 ± 1.36 F_1 = 25.50 ± 2.99 F_2 = 28.39 ± 2.34. Thus, it can be assumed that the differences in root growth that will be reported in this study were for tolerance to Al and not for root growth *per se*.

The sorghum genotypes used for this study showed wide differences for root growth in Al-toxic nutrient solutions (Table 1). The differences among sorghum genotypes for Al tolerance were in agreement with the data reported elsewhere (Borgonovi *et al.*, 1987; Furlani and Clark, 1987; Gourley, 1987). The LARL values of both Al-tolerant genotypes (IS7173 and IS12666) were several times larger than the LARL values of either of the Al-susceptible sorghum lines (Tx415 and IS1143).

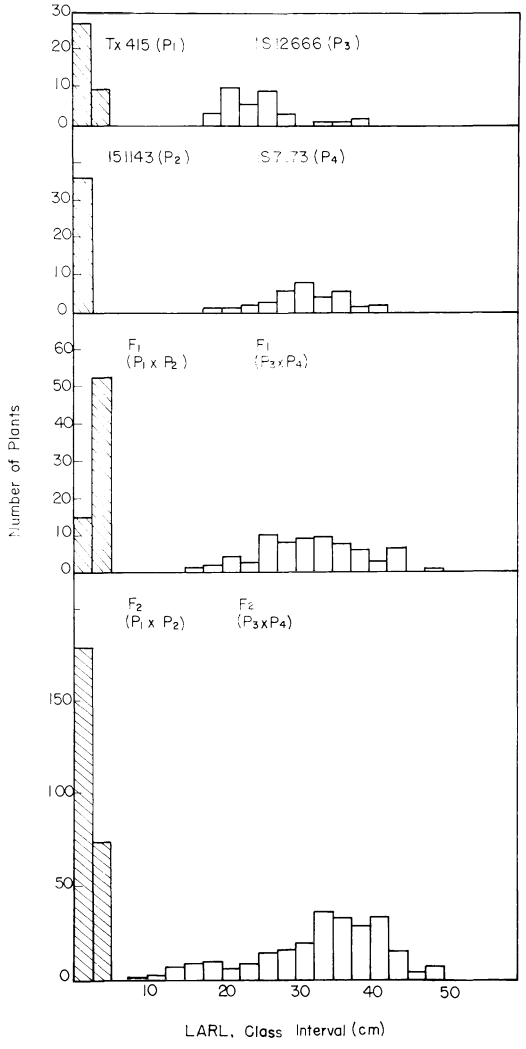

Fig. 2. Frequency distribution for LARL values in parents, F_1, and F_2 generations from the crosses Tx415/IS1143 (*left*) and IS12666/IS7173 (*right*).

Plants from F_1 families resulting from any susceptible/tolerant cross also gave LARL value several times larger than either of the Al-susceptible parents used in the cross and closer to the Al-tolerant parent, except for cross no. 4, in which the F_1 family presented a LARL that was intermediate between the parents IS1143 and IS12666. The potency ratio, an estimate of the degree of dominance, obtained for all the crosses but no. 4, was positive and different from zero, indicating that dominance plays a major role in the inheritance of Al tolerance in these crosses.

The F_2 families from all susceptible/tolerant crosses showed higher variances than parents and F_1 generations. The LARL values of the F_2 generations were lower than of the F_1 and tolerant parent but higher than the susceptible parent. Considering a LARL value of 5.0 cm as an arbitrary criterion for the differentiation of the F_2 plants into two classes of Al tolerance, the resulting segregation fitted a 3:1 ratio, typical of a single gene inheritance (Fig. 1). Thus, the parents IS7173 and IS12666 differed in their reaction to Al toxicity from the parents Tx415 and IS1143 by a pair of dominant genes. Single gene control of Al tolerance has also been suggested to occur in barley (Reid, 1971), maize (Rhue, 1979), and wheat (Kerridge and Konstad, 1968).

Broad sense heritability values for the susceptible/tolerant crosses were high indicating that selection of plants for Al tolerance should be effective. The F_1 and F_2 families from susceptible/susceptible and tolerant/tolerant crosses behave like the parents involved in the cross (Fig. 2).

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 221–225.
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PLSO PN 156

Genetics of tolerance to aluminium in wheat (*Triticum aestivum* L. Thell)

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Key words: aluminium, accumulation, aneuploid, wheat

Abstract

Preliminary studies indicated that aluminium-tolerance in wheat (*Triticum aestivum* L. Thell.) is a dominant character controlled by several genes. The present paper describes further work on localization and characterization of some of these genes in the genome of the medium Al tolerant wheat cultivar Chinese Spring (C.S.), using an aneuploid series (ditelosomics). Aluminium-tolerance of seedlings was assessed using the modified 'pulse' method; the aluminium concentration in the nutrient solution causing irreversible damage to the root apical meristems on exposure for 24 h at 25°C was the measure of Al-tolerance. At least three different factors controlling Al-tolerance in the C.S. cultivar were located on chromosomes 5As, 2Dl and 4Dl. Significant differences were found in Al-uptake and accumulation in roots of the respective ditelosomic lines and euploid seedlings of C.S. Genes controlling Al-tolerance located in the D genome (2Dl and 4Dl) were not expressed in solution culture when genes located on 5As were missing, whereas some tolerance was observed in aneuploid lines in which genes from 5As were present while genes from 2Dl and 4Dl were missing. It is concluded that Al-tolerance genes located in A genome control the expression of other Al-tolerance genes located in the D genome. The implications of the obtained results for chromosome and gene manipulations in cereals are discussed.

Introduction

Aluminium (Al) is regarded as one of the main toxic factors of acid mineral soils. A differential response of wheat cultivars to aluminium has been reported (Aniol and Kączkowski, 1979). Several attempts were made to determine the genetics of Al-tolerance in wheat. According to Kerridge and Kronstad (1968), a single dominant gene was responsible for Al-tolerance in a cross between the wheat varieties Druchamp and Brevor, but it was assumed that additional tolerance genes were present in 'Atlas 66'. The results of Iorczeski and Ohm (1974) indicated the occurrence of several different Al-tolerance genes in wheat cultivars 'IAS 58' and 'Norteno', which was consistent with Campbell and Lafever (1978, 1981) and Aniol (1984), who stated that Al-tolerance in wheat was not simply inherited and that the expression of Al-tolerance was additive with high values of heritability.

Polle *et al.* (1978) found that the substitution of chromosome 4D from the 'Thatcher' wheat cultivar into 'Chinese Spring' (C.S.) reduced the level of Al-tolerance in 'Chinese Spring' close to the level of 'Thatcher', suggesting that Al-tolerance in C.S. is located on chromosome 4D. Ditelosomic lines of C.S. were used by Aniol and Gustafson (1984a) for the localization of Al-tolerance genes in wheat. Several genes were located on chromosome arms of the A and D genomes. Takagi *et al.* (1983) found that the main genes controlling Al-tolerance in C.S. are located on the long arms of chromosomes 2D and 4D and a minor one on the long arm of chromosome 2B.

The results of further screening of C.S. aneuploid lines are presented together with the preliminary data on the biochemical expression of located genes in the 'Chinese Spring' genome.

Materials and methods

The 25 ditelosomic lines of C.S. and euploid seeds of this variety were obtained from Dr E R Sears. The Al-tolerance of tested seedlings was assessed using the nutrient culture, modified pulse method as described earlier (Aniol, 1983). The aneuploid lines were tested at three concentrations of aluminium in the nutrient medium; all concentrations were nontoxic to euploid C.S. seedlings. It was assumed that the ditelosomic lines showing no root regrowth after treatment with sublethal doses of Al, the missing chromosome arm carried a gene or genes controlling an Al-tolerance mechanism in C.S.

Selected aneuploid lines with missing chromosome segments, carrying Al-tolerance genes were tested in modified nutrient medium together with euploid C.S. seedlings. Cation concentration in the medium was increased fivefold as compared to a standard procedure in order to obtain wider differentiation of tested lines (Ali, 1973; Aniol, 1983). Aluminium concentrations in roots and root tips were estimated after ashing using the catechol violet method (Wilson, 1984).

Crossing of selected aneuploid lines with the euploid tolerant Brazilian variety BH1146 was performed in the greenhouse, the growth chamber, and the field. Chromosomes were counted in squash preparations from root meristems using the orcein method.

Results and discussion

Ditelosomic lines of 'Chinese Spring' (C.S.) wheat were screened at three sublethal Al-concentrations in nutrient solution (Table 1). Line 5Al (with missing short arm on both homologous 5A chromosomes) showed irreversible damage to

Table 1. Aluminium tolerance of Chinese Spring ditelosomic lines. Roots of 4-days-old seedlings were exposed in nutrient solution to three aluminium concentrations for 24 h at 25°C. After Al stress, seedlings were grown 48 h in the same medium without Al. The ability of roots to continue growth after Al stress was a measure of tolerance

Aneuploid line	Al-conc. in the medium in μM			Missing arm
	37	56	74	
01. Ditelo 1As	+	+	+	1Al
02. Ditelo 1Al	+	+	+	1As
03. Ditelo 2As	+	+	+	2Al
04. Ditelo 3Al	+	+	+	3As
05. Ditelo 3As	+	+	+	3Al
06. Ditelo 4Al	+	+	+	4As
07. Ditelo 5Al	−	−	−	5As*
08. Ditelo 5Al	+	+	+	6As
09. Ditelo 7As	+	+	+	7Al
10. Ditelo 7Al	+	+	+	7As
11. Ditelo 1Bl	+	+	+	1Bs
12. Ditelo 2Bl	+	+	+	2Bs
13. Ditelo 3Bl	+	+	+	3Bs
14. Ditelo 4Bl	+	+	+	4Bs
15. Ditelo 5Bl	+	+	+	5Bs
16. Ditelo 6Bs	+	+	+	6Bl
17. Ditelo 6Bl	+	+	+	6Bs
18. Ditelo 1Ds	+	+	+	1Dl
19. Ditelo 1Dl	+	+	+	1Ds
20. Ditelo 2Ds	+	+	−	2Dl*
21. Ditelo 3Dl	+	+	+	3Ds
22. Ditelo 4Ds	+	+	−	4Dl*
23. Ditelo 4Dl	+	+	+	4Ds
24. Ditelo 5Dl	+	+	+	5Ds
25. Ditelo 6Dl	+	+	+	6Ds
26. C.S.	+	+	+	-

+ = roots able to regrow after Al stress.
− = roots with irreversible damage to meristems.

root apical meristems after exposure to all three Al concentrations used. Root apical meristems of both other 'critical' aneuploid lines (2Ds and 4Ds) were damaged only at highest Al concentration in the medium, while roots of euploid C.S. seedlings were not damaged at all.

The above results indicate that genetic factors controlling Al-tolerance in C.S. wheat are located on the short arm of chromosome 5A and on the long arms of chromosomes 2D and 4D. However, genes located on the D genome were expressed only at higher Al-concentration in the medium, while genes located on 5A chromosome were expressed at all Al-concentrations used. It can be concluded that genetic factors located on the short arm of chromosome 5A are the major ones, since aneuploid lines without this chromosome fragment show a complete lack of Al-tolerance despite the fact that genes located on 2Dl and 4Dl chromosomes are present.

The critical aneuploid lines: 5Al, 2Ds and 4Ds and some other aneuploid lines of C.S. together with euploid C.S. were crossed with very tolerant Brazilian wheat BH 1146. Root apical meristems of BH 1146 seedlings were approximately 5-times more tolerant to aluminium ions than C.S.; irreversible damage of root meristems was observed after 24 h incubation in medium containing 111 μM Al and 593 μM Al in seedlings of C.S. and BH 1146, respectively (Aniol, 1983). F_2 seedlings were tested at 148 μM Al in the medium, *e.g.* slightly above the toxicity level for C.S. It was assumed that at this Al concentration, all degrees of Al-tolerance introduced from tolerant parent into a hybrid would be identified (Table 2). The frequency of Al tolerant seedlings in the F_2 population from crosses between ditelo 5Al and 4Ds aneuploid lines and BH 1146 markedly differ from the frequency obtained in crosses of other aneuploid lines and euploid C.S. with BH 1146; approximately 50% less tolerant seedlings were found in F_2 hybrids from C.S. 'critical' aneuploid × BH 1146. Surprisingly, segregation in F_2 population involving 2Ds ditelo line did not differ from the one obtained in the control F_2 populations. It can be concluded that the results obtained confirm the presence of genes controlling Al-tolerance in C.S. on short arm of chromosome 5A and on the long arm of chromosome 4D.

A significantly higher frequency of tolerant seedlings was found among F_2 seedlings from the cross ditelo 6Bl × BH 1146. This might suggest that some factors suppressing Al-tolerance are located on the short arm of 6B chromosome of C.S.

It can be concluded that besides the major genetic factors controlling Al-tolerance in C.S., located on short-arm of chromosome 5A and on the long arm of chromosome 4D some other genetic factors modifying the expression of this gene are present in the genome of C.S. and BH 1146.

An attempt was made to identify the physiological expression of Al-tolerance genes located in C.S. genome by analyzing Al accumulation in roots of seedlings exposed to Al ions (Table 3). Under the modified conditions of growth and Al-concentration, root apical meristems of seedlings ditelo 5Al

Table 2. Aluminium tolerance of F_2 seedlings from crosses of C.S. ditelosomics and tolerant BH 1146. Seedlings were screened at 148 μM of Al for 24 h at 25°C. Data are means of at least three independent experiments

Aneuploid line	# of tested F_2 seedlings	Percent of tolerant seedlings (± S.D.)
1Al	246	51 ± 13
2As	170	43 ± 12
3Al	467	53 ± 11
4Al	406	46 ± 5
5Al	597	20 ± 9*
6Al	725	52 ± 13
6As	202	47 ± 12
3Bl	440	50 ± 6
6Bs	489	51 ± 10
6Bl	154	75 ± 6*
2Ds	170	54 ± 12
4Ds	423	18 ± 12*
4Dl	681	57 ± 12
C.S.	425	42 ± 9

Table 3. Aluminium concentration in roots and root tips from tolerant and sensitive C.S. lines. Aluminium concentration is expressed in mg Al g dry wt^{-1}. Root tips constitute 20 ± 3.3% of total root dry wt

Line		Al-conc. in the medium in μM			
		74	148	222	296
C.S.	Roots	0.53	0.69	0.64	0.78
	Tips	1.90	1.89	3.77	5.17*
5Al	Roots	0.72	0.92	0.91	1.29
	Tips	3.20*	3.48*	4.20*	5.43*
2Ds	Roots	0.52	0.50	0.59	0.92
	Tips	1.35	2.21	2.59	5.47*

* = irreversible damage to apical meristems.

Table 4. Aluminium accumulation in roots and root tips of tested C.S. lines. Al accumulation expressed in micrograms of Al/100 seedlings

Line	Al-conc. in the medium in μM							
	74		148		222		296	
	Roots	Tips	Roots	Tips	Roots	Tips	Roots	Tips
C.S.	64.5	49.1	85.3	71.8	110.7	94.7	143.8	132.5
% in tips		76		84		86		92
5Al	99.6	95.1	112.3	102.4	126.2	120.9	173.5	161.1
% in tips		95		91		96		93
2Ds	59.7	37.3	75.6	64.2	91.5	79.8	144.1	130.3
% in tips		62		85		87		90

were damaged at all tested concentrations of Al in the medium, while roots of ditelo 2Ds and C.S. euploid seedlings were damaged only at highest Al concentration in the medium. These physiological differences were reflected in Al accumulation in the roots, particularly in the root tips, where 85–95% of total Al found in roots was accumulated (Table 4). Approximately 100% more aluminium was accumulated in root tips of ditelo 5Al seedlings at 75 μM Al in the medium than in root tips of another ditelosomic line and euploid C.S., while root tips of 2Ds ditelo line accumulated less Al than euploid C.S. These differences in Al accumulation disappeared at highest external Al concentration (296 μM) where roots of all tested genotypes were irreversibly damaged. It is important to note that root tips of tolerant euploid C.S. seedlings accumulated more Al at 222 μM external Al (3.77 mg Al per g dry wt.) than root tips of ditelo 5Al line at 75 μM Al in the medium, and despite this high Al content, roots of C.S. were able to grow while roots of ditelo 5Al were irreversibly damaged.

One can conclude that the absence of genes controlling Al tolerance located on 5As chromosome in C.S. is manifested by increased Al uptake by root tips at low external Al concentration. Consequently, Al accumulation in root tips is faster in sensitive than in tolerant lines, leading to destruction of root meristems at lower external Al concentrations, or shorter times of exposure to toxic ions. But also it is evident that root tips of tolerant lines can survive, at least temporary, higher internal concentration of aluminium than roots of sensitive genotypes. One can speculate that genes located on 5As chromosomes control the mechanism of Al-uptake, as do genes located on 2Dl chromosome, but additional genes from the 5A chromosome are also responsible for some mechanism of Al detoxification inside root tissue. Various mechanisms of Al detoxification have been postulated: binding of Al by mucilages produced by roots (Horst *et al.*, 1982), changes of pH in rhizosphere (Foy *et al.*, 1978) as well as chelation of Al by organic acids (Foy *et al.*, 1978) or metal binding proteins (Aniol, 1984). Further work on isolation and identification of the mechanism of located Al-tolerance genes in wheat is needed.

The results indicate that aluminium tolerance in wheat is a complex character, controlled by several major genes, minor modifying genes, and probably by genes controlling suppression of Al-tolerance genes. This information is important for breeders trying to incorporate Al-tolerance into wheat varieties as well as for programs aimed at breeding of Al tolerant varieties of wheat-rye hybrids (triticale). It can be concluded that incorporation of alien variation from related species into cereals would not be a simple process.

Acknowledgment

The partial support of this work by USDA grant FG-Po-369/PL-ARS-118 is gratefully acknowledged.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 227–230.
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PLSO PN 157

The uptake of trace elements by spinach and bean varieties of different root parameters

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Key words: beans, micronutrients, nutritional efficiency, root parameters, spinach, varietal differences

Abstract

The uptake by plants of trace elements from the soil depends to a large extent on root characteristics and activities. Differences between plant species and varieties in the uptake of trace elements are well known. Less understood, however, are the mechanisms governing these differences and the relative significance of various root parameters.

Spinach and bean varieties were, therefore, compared with respect to their root lengths and number of root apices, and to the uptake of Mn, Fe and Zn from soil-sand mixtures. The results showed significant differences among the varieties tested, both in root characteristics and in the uptake of trace elements. However, no relationships were evident between the trace element uptake by spinach varieties and their root characteristics. Contrary to this the Fe-uptake by bean varieties exhibited a clear dependence on the total number of root apices. The uptake of Mn and Zn showed a similar relationship with some exceptions. Whether the apical regions of individual roots are the most active sites of uptake or rather affect the solubility of trace elements will be the subject of further investigations.

Introduction

Plants need micronutrients in small amounts, *i.e.* in the $\mu g\,g^{-1}$ range. Relative to these low requirements, most soils are reasonably well supplied. However, when trace element uptake by plants is not adequate to sustain their growth, it is often the availability rather than the abundance in soil which limits micronutrient uptake (Lindsay, 1974). Under these circumstances, the micronutrient uptake depends largely on root activities which affect their solubility (Godo and Reisenauer, 1980), as well as on root characteristics which control the uptake rate (Barber, 1984).

In the last years much effort has been devoted to clarifying root activities which are induced by low micronutrient availability (*e.g.* Brown and Ambler, 1973; Marschner, 1986; Römheld and Marschner, 1982; 1983). However, little attention has been paid to other root properties which may be of importance for the uptake of micronutrients. For example, little is understood about the significance of the root amount, the magnitude of the physiologically active root part (Ward *et al.*, 1978) or the specific role of root apices (Clarkson and Sanderson, 1978) in micronutrient uptake. Preliminary comparisons of spinach and bean varieties showed significant differences in root length and root morphology. In the present study, spinach and bean varieties were, compared with respect to their morphological root characteristics and their uptake of Fe, Mn and Zn from soil-sand mixtures.

Material and methods

The soil used was a chernozem with pH 7.4, $CaCO_3$ 2.5%, and organic carbon 1.5%. The soil was fertilized with 0.18 g N as NH_4NO_3, 0.2 g K as K_2SO_4, 0.12 g P as KH_2PO_4 and 0.10 g Mg as $MgSO_4$ per kg, mixed with quartz sand (1:5) and

filled into 3 kg plastic pots. Four varieties each of spinach (*Spinacia oleracea*) and beans (*Phaseolus vulgaris* L.) were chosen which cover the whole range of root lengths observed in preliminary screening. Ten seeds were planted 1.5 cm below the surface and the pots were kept under greenhouse conditions. Seven days after emergence the plants were thinned to 4 per pot.

At an age of 17–23 days, the roots from 3 pots were separated by washing them out from the soil and used for root measurements according to Helal and Sauerbeck (1986). At the end of the experiment the plant tops were cut about one cm above the soil surface and dried at 70°C. One gram of dry ground plant material was ashed at 490°C over night and the ash dissolved in 0.2 *M* HCl. P was determined photometrically as phosphomolybdate and Fe, Mn and Zn by atomic absorption. N was analysed after Kjeldahl digestion. Most of the results are means of 6 replicates.

Table 1. Dry matter yields and nutrient concentrations of 43-day old plants of various bean varieties

	Andreas	Daisy	Goldimmens	Saxa	$SD_{0.05}$
Dry wt (g/plant^{-1})	2.11	2.02	1.94	1.80	0.19
Nutrient concn.:					
P (mg g^{-1})	3.22	2.41	3.80	2.86	0.32
N (mg g^{-1})	4.24	3.65	3.82	4.80	0.48
Fe (μg g^{-1})	152	94	77	81	14.0
Mn (μg g^{-1})	41	63	44	34	9.0
Zn (μg g^{-1})	53	69	22	26	8.0

Results and discussion

As indicated in Tables 1 and 2, there were significant differences among the tested varieties with respect to root characteristics, nutrient concentrations and nutrient uptake. The range of differences in total root length between varieties reached 49%. Compared to this the number of individual root members varied up to 87%. This demonstrates varietal differences in the mean length of individual roots as well.

Even larger were the varietal differences in micronutrient uptake. The relatively highest variability was shown for Zn, the uptake of which ranged from 43 to 112 μg plant^{-1}, corresponding to a difference of 160%. The varietal differences in Fe and Mn uptake reached 120% and 108%, respectively. A comparison between root characteristics and utpake data of the various varieties reveals no simple dependence of micronutrient uptake on the overall root length. It is not surprising, therefore,

Table 2. Root characteristics and micronutrient uptake by 42-day-old plants of various bean varieties

		Andreas	Daisy	Goldimmens	Saxa	$SD_{0.05}$
Total root length (m plant^{-1})		19.6 (149)	13.2 (100)	19.4 (147)	14.1 (107)[a]	2.5
Number of roots plant^{-1}		1580 (187)	1210 (143)	1088 (129)	846 (100)	180
Micronutrient uptake						
Fe	μg plant^{-1}	321 (220)	190 (130)	149 (102)	146 (100)	35
	μg m root^{-1}	16.4 (213)	14.4 (187)	7.7 (100)	10.4 (135)	
	ng r. apex^{-1}	203 (148)	157 (115)	137 (100)	173 (126)	
Mn	μg plant^{-1}	87 (143)	127 (208)	85 (139)	61 (100)	16.0
	μg m root^{-1}	4.4 (102)	9.6 (223)	4.4 (102)	4.3 (100)	
	ng r. apex^{-1}	55 (100)	105 (191)	78 (142)	72 (131)	
Zn	μg plant^{-1}	112 (260)	79 (184)	43 (100)	47 (109)	14.0
	μg m root^{-1}	5.7 (259)	6.0 (273)	2.2 (100)	3.3 (150)	
	ng r apex^{-1}	71 (178)	65 (163)	40 (100)	56 (140)	

[a] Figures in bracket are relative values.

that expressing micronutrient uptake on a per unit root length basis does not reduce the varietal variability but may even increase it.

Contrary to this, the data in Table 2 reveal a clear relationship between micronutrient uptake and the number of individual roots. This applies especially to the Fe-uptake. An expression of Fe-uptake/root, therefore, reduces its variability from 120% to 48%. However, this 48% difference was still significant and demonstrates an additional source of variability between varieties which is not accounted for by the differences in root number.

The data in Table 2 show similar relations between the Mn and Zn uptake of various varieties on the one side and their root numbers on the other, however, with some exceptions. An example is the low Mn-uptake by the variety Andreas relative to its number of roots. The relatively high Fe-uptake of this variety suggests an Fe-Mn interaction (Toth and Romney, 1954). Similarly, a Zn-P interaction may explain the relatively low Zn-uptake by the variety Goldimmens (Boawn and Brown, 1968; Wallace *et al.*, 1978).

Tables 3 and 4 show significant differences also among spinach varieties in root morphology, micronutrient contents and trace element uptake. Contrary to the beans, however, the results show no clear-cut relationship between micronutrient uptake and morphological root characteristics. The lack of dependence of micronutrient uptake on root amount suggests that the transport to the root surface is not the limiting step. Further work on the concentration of micronutrients in the rhizosphere soil solution (Geering and Hodgson, 1969) is required to corroborate this interpretation.

Finally, Table 5 compares beans and spinach with respect to their root amount per unit of shoot dry weight, and to micronutrient uptake rate per unit root length. From this it is evident that spinach roots are much smaller but considerably more efficient than bean roots are. The mean bean root length was about 16 times larger and had 5 times more apices than of spinach plants. Nevertheless the uptake rate of micronutrients by spinach roots was 41–64 times higher than that of beans.

In cases where the micronutrient concentration of the rhizosphere soil solution is not limiting their uptake, physiological root parameters such as the maximum influx rate will be the most important. Sauerbeck and Helal (1988) discussed the factors

Table 3. Dry matter yields and nutrient contents of 35-day-old plants of various spinach varieties

	Monnopa	Vital	Matador	Atlanta	$SD_{0.05}$
Dry wt (g/plant^{-1})	1.46	1.24	1.30	1.08	0.31
Nutrient concn.:					
P mg g^{-1}	4.82	2.64	2.95	2.49	0.41
N mg g^{-1}	5.04	4.26	5.48	4.12	0.72
Fe μg g^{-1}	196	236	288	145	34
Mn μg g^{-1}	149	184	190	122	28
Zn μg g^{-1}	61	89	86	95	16

Table 4. Root characteristics and micronutrient uptake by 35-day-old plants of various spinach varieties

	Monnopa	Vital	Matador	Atlanta	$SD_{0.05}$
Total root length mm plant^{-1}	812 (193)	631 (150)	766 (182)	420 (100)	86
Number of roots plant^{-1}	242 (252)	184 (192)	146 (152)	96 (100)	34
Micronutrient uptake (μg plant^{-1})					
Fe	286 (182)	293 (187)	373 (238)	157 (100)	38
Mn	218 (165)	228 (173)	247 (187)	132 (100)	27
Zn	89 (100)	110 (124)	112 (126)	103 (116)	18

Table 5. Root characteristics and nutrient uptake by beans in comparison to spinach[a]

	Beans (B)	Spinach (S)	S/B
Root length m g shoot^{-1}	8.43	0.52	$\frac{1}{16}$
Number of roots g shoot^{-1}	599	132	$\frac{1}{5}$
Micronutrient uptake μg m root^{-1} day^{-1}			
Fe	0.29	12.0	41
Mn	0.14	9.0	64
Zn	0.10	4.8	48

[a] Mean values of 4 varieties.

controlling the significance of individual soil and root parameters for nutrient uptake. Such parameters should be investigated if the basis of the high micronutrient efficiency of spinach roots is to be better understood.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 231–236.
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PLSO PN 158

Manganese toxicity in sunflower lines

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Key words: genotypic differences, *Helianthus annuus*, manganese tolerance, manganese toxicity, sunflower

Abstract

Sunflower (*Helianthus annuus* L.) has been shown to be tolerant of high concentrations of plant-available manganese (Mn) in the root environment and high concentrations of Mn in the plant tops. This tolerance has been shown to be associated with the accumulation of excess Mn in apparently non-phytotoxic forms in and around the trichomes. However, possible genotypic variation in the tolerance of sunflower to Mn toxicity does not appear to have been studied.

Ten sunflower lines of diverse origin were grown for 21 days in solution cultures in which the Mn concentrations were maintained at 3, 30, 100 or 300 μM. All lines proved tolerant of high external and internal Mn concentrations, and plants of all lines produced the small, dark spots indicative of accumulation of Mn in the trichomes. Reductions in yield of tops and roots at 30 μM Mn were mostly small and, with two exceptions, not significant. At 100 and 300 μM, relative yields of tops were in the range of 67 to 100% and 16 to 72% of maximum, respectively. The corresponding ranges for roots were 44 to 100% and 7 to 46%. At 100 μM, Mn concentrations in tops and roots were in the range of 2920 to 3230 $\mu g\, g^{-1}$ and 1560 to 2180 $\mu g\, g^{-1}$, respectively. Relatively little of the absorbed Mn (6 to 24%) was retained in the root systems.

The data indicated marked differences among the lines in Mn tolerance, especially at 300 μM Mn. Problems in ranking lines for tolerance, and the significance of the results in terms of sunflower breeding are discussed briefly.

Introduction

Manganese (Mn) toxicity is a problem that is associated with acidic and/or poorly drained soils (Foy, 1984; Grasmanis and Leeper, 1966; Graven *et al.*, 1965). Studies with a small number of cultivars indicate that sunflower (*Helianthus annuus*) is highly tolerant of excess Mn but is susceptible to or only moderately tolerant of other soil acidity factors. Thus, in a flowing solution culture experiment in which 13 crop and pasture species were grown at a range of constant Mn concentrations, Edwards and Asher (1982) found that the critical external concentration for Mn toxicity (10% yield reduction) in sunflower cv Hysun 30 (65 μM) was the highest of any species studied. Under the conditions of the experiment, nine species had critical concentrations $< 20\, \mu M$, and 3 species $< 2\, \mu M$. In other work, it was shown that sunflower is highly susceptible to aluminium toxicity (Blamey *et al.*, 1986), and boron deficiency (Blamey *et al.*, 1987), and has only moderate tolerance to deficiencies of calcium, phosphorus, and molybdenum (Blamey *et al.*, 1987). Tolerance of low pH *per se* also appears to be low. Thus, in a flowing culture experiment with four sunflower cultivars, all died at pH 3.5, only one (Hysun 11) reached near maximum yield at pH 4.0, and two (Hysun 21, Hysun 31) required pH $\geqslant$ 5.0 for maximum yield (Blamey *et al.*, 1982). Blamey *et al.* (1986) have suggested that the above nutritional characteristics of sunflower are consistent with the evolution of the crop on neutral to alkaline soils periodically rendered Mn toxic by waterlogging.

On the basis of their observations on sunflower cv Hysun 30, Edwards and Asher (1982) concluded that the high tolerance of sunflower to Mn toxicity was due to an ability to tolerate very high concentrations of Mn in the plant tops. No evidence was found for any special ability to limit the entry of Mn into the plant, or to retain it in the root system after entry. In subsequent research with cv Hysun 31 (Blamey *et al.*, 1986), it was shown that ability to tolerate high concentrations of Mn in the tops was associated with the accumulation of excess Mn, apparently in a non-toxic form, in and around the trichomes. More recently, excretion of oxidized Mn around the trichomes has been postulated as a tolerance mechanism in cucumber (*Cucumis sativus*) (Horiguchi, 1987), and a published photograph of Mn toxicity symptoms in watermelon (*Citrullus lanatus*) (Elamin and Wilcox, 1986) suggests a similar mechanism in that species also.

As far as we are aware, there are no published reports in which differences in tolerance to Mn toxicity among sunflower cultivars and breeding lines have been examined. The present paper reports results of a preliminary investigation in which the Mn responses of ten sunflower lines of diverse origin were compared.

Materials and methods

Lines

The lines used differed widely in maturity rating and included commercial single and three-way cross hybrid cultivars, imbred male and female breeding lines, and a composite population of male lines (Table 1).

Table 1. Sunflower lines used in the experiment

Line	Maturity	
R line popn	Medium	Intercrossed composite of USA male lines
Hysun 33	Medium–Late	Single cross, Argentine × USA
HA89	Late	Inbred female line, USA
Suncross 40R	Late	Single cross, proprietary parents
Hysun 32	Medium	Single cross, USA × USA, high oil cv.
Suncross 20R	Early	Single cross, proprietary parents
Hysun 22	Early	3-way cross, Argentine × USA
Suncross 25	Early–Medium	Sister line 3-way cross, proprietary parents
Dynamite	Medium	Single cross, USA × USA
RHA274	Medium	Inbred male line, USA

Plant culture

Seeds were surface-sterilized with 1.25% (w/v) sodium hypochlorite for 10 min, washed four times with deionized water, then germinated at 25°C between paper towels and moistened with a solution containing 1000 μM calcium sulfate and 3 μM boric acid. After 3 days, uniform sets of seedlings of each line were transplanted into 480-litre culture tanks in a glasshouse. The nutrient solution in these tanks was continuously circulated with a fibreglass pump, and maintained at approximately pH 5 and 22°C. There were four single-plant replicates of each sunflower line in each tank.

The composition of the basal nutrient solution was as follows (μM): Cl 897, Ca 562, N 310, K 94, Mg 29, S 29, Fe(EDTA) 15, P 10.5, B 5, Mn 3, Zn 0.01, Cu 0.004, and Mo 0.001. In the Mn toxicity treatments, sufficient manganese sulfate was added to the tanks over a 3-day period, commencing 7 days after transplanting (Day 7), to raise the Mn concentrations to 30, 100 or 300 μM. Manganese concentrations were checked by AAS twice weekly and adjusted as necessary. The solutions were not analysed for elements other than Mn, but additional basal nutrients were added on Days 14 and 21.

Harvesting, analysis

On Day 28, the plants were separated into roots and tops, and oven dried prior to weighing. Subsamples of plant material from the 3, 30 and 100 μM Mn treatments were dry ashed at 450°C for 8 hours, and the ash was taken up in approx. 2.4 M HCl. Manganese was then determined by AAS.

Results

Symptoms of manganese toxicity

The symptoms of Mn excess in sunflower have been described recently in some detail (Blamey *et al.*, 1986). Briefly, they include darkening of the trichomes; necrotic spots on the underside of the cotyledons; irregular chlorotic or necrotic patches on the lower leaves, especially along the veins; crinkling of the older leaves; and veinal chlorosis, crinkling, and necrosis of the upper leaves. These symptoms were observed also in Mn toxic treatments the present study, their intensity and rate of development varying with sunflower line and treatment (data not presented).

On the basis of previous work, no symptoms of excess Mn in the root environment were expected at 3 μM Mn, but under the conditions of the experiment, all lines except Suncross 20R showed slight darkening of the trichomes on the older plant parts by Day 28. In addition, RHA274 showed some darkening of the trichomes on the younger growth, and Hysun 32 developed necrotic spotting on the underside of the cotyledons.

Dry matter yield response

Marked differences in growth rate were evident among the lines, with top yields in the 3 μM Mn treatment at Day 28 varying from 1.73 g plant^{-1} (RHA274) to 5.92 g plant^{-1} (Suncross 40R) (Table 2). However, apart from the minor symptoms noted above, all plants in the 3 μM treatment appeared healthy. Although top yields were reduced on average by approximately 4% at 30 μM Mn, and root yields by approximately 12%, except for root yields in Hysun 22 and Dynamite, none of the effects of this treatment on yield was significant ($P = 0.05$) (Table 2). Increasing the Mn concentration to 100 or 300 μM caused substantial reductions in the yields of tops and roots of all lines, the effect on roots generally being larger than on tops (Table 2).

The data reveal substantial differences among lines in tolerance to Mn toxicity. Thus, at 300 μM Mn the R line population, Hysun 33, HA89 and Suncross 40R had tops yields < 18% of maximum, whereas Dynamite had a tops yield of nearly 53% and RHA274 of approximately 72% (Table 2). Similarly, the relative yield of roots in this treatment varied across lines from 7.4% (HA89) to 45.6% (Table 2). The restricted number of Mn concentrations, and the variability of the data precluded the calculation of critical external Mn concentrations for the sunflower lines, but the data are not inconsistent with the high value of 65 μM reported previously for Hysun 30 (Edwards and Asher, 1982).

Table 2. Effect of external Mn concentration in solution culture on absolute and relative dry matter yields of tops and roots of 10 sunflower lines grown for 28 days in solution culture

Line	Tops					Roots				
	Absolute yield (g plant^{-1})	Relative yield (%)				Absolute yield (g plant^{-1})	Relative yield (%)			
	Mn concentration in solution (μM)									
	3	3	30	100	300	3	3	30	100	300
R line popn	5.40	100	88.8	87.1	16.1	1.62	100	77.0	80.4	8.8
Hysun 33	4.65	100	93.4	84.0	16.4	0.86	78.7	100	81.9	8.7
HA89	2.90	95.5	100	78.8	17.1	0.69	76.3	100	45.9	7.4
Suncross 40R	5.92	100	100	75.4	17.8	1.40	85.5	100	65.2	8.5
Hysun 32	4.50	100	96.8	74.7	28.7	1.65	100	67.9	44.2	12.4
Suncross 20R	4.82	99.0	100	69.8	42.8	1.86	100	83.5	56.7	19.3
Hysun 22	4.96	100	89.4	85.0	44.8	1.79	100	55.6	45.5	30.1
Suncross 25	4.51	93.5	100	67.7	47.3	1.24	100	100	80.6	29.4
Dynamite	4.27	100	87.6	93.1	52.7	1.61	100	57.9	59.7	29.9
RHA274	1.73	82.0	74.5	100	72.4	0.76	96.2	77.2	100	45.6
Means	4.37	96.8	93.1	81.8	35.6	1.35	93.7	81.9	66.0	20.0
LSD ($P = 0.05$)	1.15			24.1		0.65			40.8	

Manganese concentrations in tissue

At 3 μM Mn, concentrations of Mn in the tops ranged from 372 $\mu g\,g^{-1}$ (Suncross 20R) to 524 $\mu g\,g^{-1}$ (RHA274), and that in the roots from 159 $\mu g\,g^{-1}$ (Hysun 33) to 326 $\mu g\,g^{-1}$ (RHA274) (Table 3). Increasing the external Mn concentration to 30 μM caused large increases in the Mn concentrations in tops and roots of all lines (to > 1700 and $> 1200\,\mu g\,g^{-1}$, respectively), but as noted previously, yields were significantly depressed in only two cases. At 100 μM Mn, concentrations in tops and roots were further increased, the mean for tops of all lines being 3010 $\mu g\,g^{-1}$ and that for roots 1840 $\mu g\,g^{-1}$ (Table 3). In all cases except one (HA89 at 30 μM Mn), concentrations in roots were lower than in tops.

Partitioning of dry matter and manganese between roots and tops

Overall, there was a tendency for the proportion of plant dry mass represented by the root system to decline as the external Mn concentration increased, although this effect of Mn was not statistically significant in the case of some lines (Table 4). The data also reveal substantial differences among lines in the partitioning of dry matter between tops and roots. Thus, in the 3 μM Mn treatment, root systems accounted for from only 15.5% of the total dry matter (Hysun 33) to as much as 30.8% (RHA274).

With the exception of HA89 in the 30 μM treatment, the proportion of the total plant Mn retained in the root system was less than the corresponding proportion of the total dry matter (Table 4). Hence in no line was there any evidence of preferential retention of Mn within the root system.

Discussion

In the present study, all sunflower lines had high tolerance to Mn toxicity, most producing maximum or near-maximum yields at an external Mn concentration of 30 μM, and in the case of some lines, at 100 μM (Table 2). These concentrations may be compared with toxicity thresholds of 0.3 to 18 μM given for six species in a review by Asher (1978), and with critical values of 1.4 to 18 μM for nine other species reported by Edwards and Asher (1982). In the latter study, apart from sunflower (critical value 65 μM), only three species were able to tolerate Mn concentrations greater than 30 μM (*Centrosema pubescens* 51 μM, safflower (*Cathamus tinctorius*) 48 μM, and pigeon pea (*Cajanus cajan*) 46 μM).

The darkening of trichomes on all lines in the present study indicates that the local accumulation of Mn in these structures, previously shown to be a tolerance mechanism in Hysun 31 (Blamey *et al.*,

Table 3. Concentrations of Mn in tops and roots of 10 sunflower lines grown at three Mn concentrations in solution culture

Line	Mn concentration in plant tissue ($\mu g\,g^{-1}$)					
	Tops			Roots		
	Mn concentraiton in solution (μM)					
	3	30	100	3	30	100
R line popn	382	1870	2930	189	1300	2180
Hysun 33	452	2100	2960	159	1260	1560
HA89	388	1710	3080	199	1890	1580
Suncross 40R	483	2050	3040	235	1380	2100
Hysun 32	419	1990	3030	320	1530	1890
Suncross 20R	372	2030	3230	264	1750	1600
Hysun 22	430	1980	2800	263	1250	1580
Suncross 25	436	2160	2990	279	1240	1810
Dynamite	481	2000	3080	291	1570	2020
RHA274	524	2070	2920	326	1610	2050
Means	437	2000	3000	253	1480	1840
LSD ($P = 0.05$)		315			488	

Table 4. Effect of external Mn concentration on the percentage distribution of dry matter and Mn in the roots of 10 sunflower lines

Line	DM in roots (% of total)				Mn in roots (% of total)		
	Mn concentration in solution (μM)						
	3	30	100	300	3	30	100
R line popn	22.6	20.1	21.6	13.5	13.1	14.9	17.6
Hysun 33	15.5	19.7	18.6	10.7	6.0	13.1	10.7
HA89	18.8	22.5	14.5	11.4	10.5	24.4	7.6
Suncross 40R	18.5	21.6	19.0	12.3	10.0	15.8	13.9
Hysun 32	26.5	20.8	17.7	13.9	21.7	16.6	11.8
Suncross 20R	27.9	24.1	23.9	14.1	20.9	21.5	14.3
Hysun 22	26.7	18.3	16.1	19.4	17.4	12.9	10.0
Suncross 25	21.8	19.5	20.6	13.4	15.1	12.9	13.5
Dynamite	26.2	19.4	18.6	17.6	18.6	16.4	13.0
RHA274	30.8	27.4	27.0	19.1	21.4	22.2	20.3
Means	23.5	21.3	19.7	14.5	15.5	17.1	13.2
LSD ($P = 0.05$)			7.5			8.5	

1986), is a general tolerance mechanism in sunflower. As in earlier work with Hysun 30, there was no evidence that any sunflower line tolerated high external Mn concentrations by immobilising Mn in the root system as has been reported for wheat (*Triticum aestivum*) (Horiguchi, 1987). Although Mn uptake rates were not measured in the present study, the high Mn concentrations in the tissues of all lines suggests that, as in earlier work with Hysun 30 (Edwards and Asher, 1982), ability to limit uptake at high external Mn concentrations was not an important tolerance mechanism.

At present, there is no generally accepted method of ranking species or cultivars for tolerance to heavy metal toxicities. Often, single indices such as critical external concentration (10% or 50% yield reduction) or relative yield at a particular level of supply of the heavy metal have been used. However, as Berry and Wallace (1981) have pointed out, rankings may change depending on the particular level of toxicity selected, due to crossing over of response curves once the threshold concentration for toxicity has been exceeded. Hence they suggest that the whole response curve needs to be considered, rather than a single point on the curve. Unfortunately, their proposal to characterize tolerance by estimating both the threshold concentration for toxicity, and the rate of decrease in yield beyond this concentration, does not yield a simple index by which genotypes can be ranked.

In the present study, despite the high Mn tolerance of all lines, there was clear evidence of differences in tolerance among lines, particularly at 300 μM Mn. In the latter treatment, relative yields of tops ranged from 16% of maximum (R line population) to 72% (RHA274), and those of roots from 7% (HA89) to 46% (RHA274) (Table 2). Although there were significant differences in relative yield amongst the cultivars, neither the use of the l.s.d., nor Duncan's multiple range test, separated the lines into distinct groups in terms of performance at 300 μM Mn. However, application of a cluster analysis technique (McLachlan and Basford, 1987) to the data for the 300 μM treatment divided the lines into two groups. On the basis of relative tops or whole plant yields, the R line population, Hysun 33, HA89, Suncross 40R, and Hysun 32 formed a susceptible group with an expected mean relative yield of 19.4% (17.5% for whole plant), while the remaining lines formed a tolerant group with which an expected mean yield of 51.9% (46.7% for whole plant). Application of the same technique to the root data at 300 μM yielded the same grouping of lines except that Suncross 20R moved from the tolerant to the susceptible group of lines.

However, relative yields of tops and roots at 300 μM were not significantly correlated with the corresponding values at 100 μM ($r = 0.42$, 0.34, respectively). Thus, grouping of line according to tolerance at 300 μM Mn may not indicate tolerance at lower Mn concentrations. To overcome this problem, the cluster analysis technique (McLachlan and Basford, 1987), was applied to the complete data set for tops yield (ten lines $\times$ four Mn treat-

ments). This technique identified RHA274 as overall the most tolerant line followed by Dynamite and Hysun 22. The relatively poor growth of Suncross 25 at 100 μM Mn was responsible for its exclusion from the second group. The next most tolerant group contained Suncross 25, Suncross 20R, Hysun 33, and the R line population. The least tolerant group contained Hysun 32, Suncross 40R, and HA89. Although the R line population and Hysun 33 made the poorest growth of all the lines at 300 μM Mn, the analysis did not place them in the most susceptible group, because of their relatively good growth at 100 μM. The results suggest that cluster analysis could be a useful tool in the analysis of data concerned with genetic differences in tolerance to adverse nutritional conditions.

The existence of variation among sunflower lines in tolerance to excess Mn, suggests that it may be possible to manipulate this characteristic using crop improvement techniques. However, the usefulness of such an approach is limited by the fact that Mn tolerance in sunflower is already very high in comparison with other crop species, and is not accompanied by tolerance to other acid soil infertility factors. Hence, genetic manipulation of Mn tolerance in sunflower would seem to be justified only if Mn excess was identified as an important constraint to production of the crop on non-acidic soils of high Mn status.

Acknowledgements

The authors wish to thank Dr D George of the Queensland Department of Primary Industries for supplying seed of the sunflower lines used in this study.

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PLSO PN 159

Electrophysiological membrane characteristics of the salt tolerant *Plantago maritima* and the salt sensitive *Plantago media*

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Key words: ion permeability, membrane conductance, membrane potential, microelectrode, Nernst potential, plant roots

Abstract

Membrane potential, electrical conductance and plasmamembrane permeability for Na^+, K^+ and Cl^- of root cells of the salt tolerant *Plantago maritima* and the salt sensitive *P. media* were compared. Plants were grown with (25 mol m^{-3}) or without NaCl. No differences were found in the membrane potentials between the species when measured in growth medium, either plus or minus NaCl. When plants were grown in 25 mol m^{-3} NaCl membrane potentials in both species were slightly more positive.

Na and K ions both caused depolarization of the membrane potential. These were not equal for the two species, depolarization caused by K^+ ions was about 3.5 times greater than that caused by Na^+ ions. Membrane permeability for both ions seemed less in the salt tolerant species. Passive Na^+ permeability, *i.e.* in the presence of the uncoupler CCCP, was about 25% of the K^+ permeability in *P. media*, and 60% in *P. maritima*. From comparison of permeabilities under 'active' and 'passive' conditions and the related ion concentrations in the root cells it was concluded that in both species active, *i.e.* against the electrochemical gradient, Na^+ efflux pumping must exist at the cortex medium boundary.

Introduction

Salt tolerance is a gradual rather than a principal difference between plants. (Flowers *et al.*, 1977; Greenway and Munns, 1980; Walker, 1986). Many questions remain concerning the mechanism which enables one species to grow at salinities of 500 mol m^{-3} while others die at 25 mol m^{-3}. The main characteristic of salt tolerance may be the ability to acquire sufficiently cheap osmotic ions, in order not to suffer from drought stress, and still remain a strong coupling between growth rate and ion uptake, the latter to avoid cytoplasmic toxification from excessive ion influx (Flowers *et al.*, 1977; Stelter and Jeschke, 1983; Yeo and Flowers, 1986).

Membrane characteristics of a salt tolerant Plantago (*P. maritima*), which is able to grow in NaCl concentrations up to 300 mol m^{-3}, were compared with the salt sensitive *P. media*, which has an extensive growth rate reduction at 25 mol m^{-3} (results not shown), (Erdei and Kuiper, 1979).

Earlier research showed that at low salinity *P. media* is a sodium excluder, preferably transporting potassium ions to the shoot while *P. maritima* is able to accumulate sodium, when no more than 20–25 mmol m^{-3} NaCl is present. These characteristics are caused by deviating K^+/Na^+ selectivities. (De Boer, 1985; Tanczos *et al.*, 1980).

One of the driving forces for the transport of ions from cortex to the shoot symplasm is the trans root potential (TRP), *i.e.* the potential difference between the external medium and xylem sap (Graham and Bowling, 1977). As does the membrane potential, the TRP has a passive (diffusional) and an active (electrogenic) component. The latter resulting from the 'back to back' arranged plasmalemma proton pumps situated at the cortex medium and the xylem root-parenchym interfaces

(De Boer and Prins, 1984; De Boer *et al.*, 1983; Hanson, 1978).

Obviously differences in K^+/Na^+ selectivity, and/or transport-characteristics between salt sensitive and salt tolerant species must, at least partly, originate from differences in ion permeabilities and pump characteristics on the medium cortex boundary, the xylem root-parenchym boundary or both (Moon *et al.*, 1986; Stelter and Jeschke, 1983; Walker, 1986).

Materials and methods

Plant material

Seeds of *Plantago maritima* and *Plantago media* were germinated on vermiculite in the greenhouse. Fourteen-day-old seedlings were transferred to climate chambers with the following regime: a 12 hr light period, 58 $W m^{-2}$, 60% r.h., day and night temperature 20 and 18°C (De Boer, 1985). Plants were grown in 30-L/containers on 25% Hoagland nutrient solution. The solution was renewed twice a week. Due to impurities NaCl concentrations were 20 $mmol m^{-3}$ when no NaCl was added. 25 $mol m^{-3}$ NaCl was used to grow plants under saline conditions.

Electrophysiology

Electrodes were pulled from borosilicate glass capillaries and filled with 3 *M* KCl, pH 2. Resistances varied between 5 and 15 M ohm. Distal root segments, 3–4 cm long, diameter 150–300 μm, were mounted in a glass cuvet. Membrane potential measurements of root cortex cells were carried out in growth medium unless mentioned otherwise.

Ion analysis

The root free space was exchanged with 10 m*M* $CaSO_4$ (10 minutes, 20°C) in order to remove absorbed Na^+, K^+ and Cl^- ions. Root fresh and dry weights were determined and dried roots were incubated in 10 m*M* $CaSO_4$ for one hour to exchange internal ion contents. K^+ and Na^+ ions were determined with atomic absorption spectrophotometry, Cl^- was determined by Ag titration. Concentrations are expressed on a tissue water basis.

The membrane potential

Under 'active' conditions this can be thought of as the result of two parallel current sources according to the equation:

$$E_m = \frac{E_p G_p + E_d G_d}{G_p + G_d} \quad (1)$$

in which E_p and E_d are the electrogenic and diffusional electro motive forces, Gp and Gd the respective conductances (Spanswick, 1981). In the presence of CCCP the electrogenic part of the E_m becomes zero; respiration is uncoupled and CCCP acts as a protonophore shunting electrogenic proton transport. This leaves E_m existing of a diffusion potential only, and so will be referred to as E_m under 'passive' conditions.

Ion permeabilities (passive)

They were determined using the Goldman equation:

$$E_m = 60 \log \frac{[K^+]_o + \alpha[Na^+]_o + \beta[Cl^-]_i}{[K^+]_i + \alpha[Na^+]_i + \beta[Cl^-]_o} \quad (2)$$

in which ó and i are outside and inside ion activities, α and β are the relative passive ion permeabilities for Na^+ and Cl^-, compared to K^+. CCCP (20 $mmol m^{-3}$) was used as uncoupler of electrogenic proton transport.

Ion permeabilities (active)

Depolarization of the membrane potential caused by addition of potassium or sodium salts was taken as a measure of ion permeability under 'active' conditions (*i.e.* without inhibition of electrogenic transport). Depolarization is a function of pump capacities and passive permeabilities. (The latter is, together with the diffusion gradient, responsible for E_d and G_d in Eq. 1). To standardise depolarization, values were corrected for differences in the diffusion gradients with a factor log([in]). Cholinechloride, K^+ MES and Na^+ MES were used

to check the influence of chloride on the membrane potential.

Active sodium efflux pumping

Active sodium efflux (*i.e.* against the electrochemical gradient) was tested by adding 50 mol m^{-3} NaCl and 20 mmol m^{-3} CCCP to the growth medium to one set of plants, while control plants were placed in growth medium with only NaCl. Plants were then incubated for 6 hours in the climate chamber after which ion contents (K^+, Na^+ and Cl^-) of roots and shoots were determined.

Results

Ion activities

Table 1 shows the ion concentrations in the root and leaf tissue. Under non saline conditions *P. media* had no detectable amount of Na^+ in roots and shoot but *P. maritima* was able to accumulate Na^+ in spite of its low concentration (*ca.* 20 mmol m^{-3}, due to impurities). Grown under saline conditions with 25 mol m^{-3} NaCl this characteristic reversed, resulting in higher Na^+ influx in *P. media* than in *P. maritima*. Both species tend to keep their Na^+ levels low in the root cells (De Boer, 1985; Erdei and Kuiper, 1979; Tanczos *et al.*, 1980).

Membrane potentials

From Figure 1 it can be seen that membrane potentials (E_m) measured in growth medium are comparable for both species, varying between -115 to -120 mV. When the external K^+ ion concentration was increased E_m rapidly depolarised in both species. In *P. media* the measured membrane potential coincides with the K^+ Nernst potential, calculated using tissue ion activities. Addition of Na^+, also depolarised E_m but to a much lesser extent. K^+ ions at a concentration of 25 mol m^{-3} resulted in 52–67% depolarisation of E_m; Na^+ at the same concentration only caused 13–25% depolarization. Figure 1 also shows that the overall membrane conductance (composed of passive permeabilities and pump capacities) in *P. maritima* was less than in *P. media*.

Table 1. Ion concentrations of *P. maritima* and *P. media* in roots and shoots. Plants were grown for 7–8 weeks, without (0) or with 25 mol m^{-3} NaCl (25) added to the growth medium. Concentrations are expressed as mol m^{-3} on tissue water basis

Species		Roots			Shoots		
		K^+	Na^+	Cl^-	K^+	Na^+	Cl^-
P. media	(0)	107	< 0.5	< 0.5	149	< 0.5	< 0.5
	(25)	99	36	39	113	218	98
P. maritima	(0)	85	4.5	< 0.5	124	11	9
	(25)	90	54	37	81	155	61

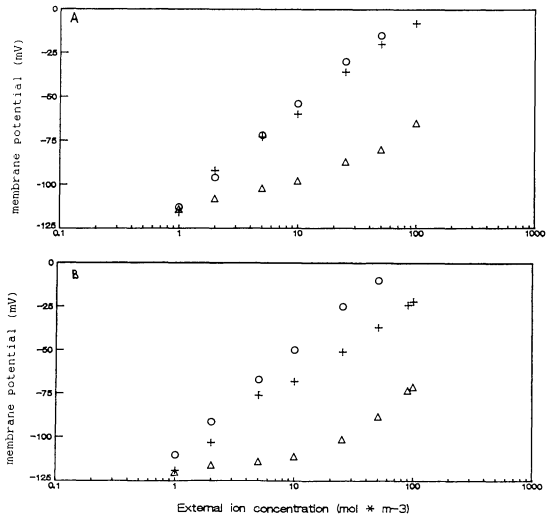

Fig. 1. Membrane potentials in root cortex cells of *P. maritima* (**A**) and *P. media* (**B**). E_m was measured in a growth medium in which the external cation concentration was increased with NaCl or KCl. +, KCl; △, NaCl; ○, K-Nernst.

Passive specific ion permeabilities

After addition of the uncoupler carbonyl cyanide chlorophenylhydrazone (CCCP, 20 mmol m^{-3}) E_m depolarised about 40 mV (results not shown). Using tissue concentrations (Table 1) in the Goldman equation, relative permeabilities for Na^+ and Cl^- (α and β) were determined for plants grown under saline and non saline conditions (Table 2). Apparently the relative Na^+ permeability (α) in *P. maritima* is higher than in *P. media*; α

Table 2. Specific Na^+ and Cl^- permeability, relative to K^+, in root cortex cells of *P. maritima* and *P. media*. Plants were grown without (0) or with 25 mol m^{-3} NaCl (25) added to the growth medium

Species		Rel. Na^+ perm.	Rel. Cl^- perm.
P. media	(0)	0.25	0.07
	(25)	0.17	0.10
P. maritima	(0)	0.58	0.11
	(25)	0.32	0.08

decreased under saline conditions. Cl^- permeability (β) was relatively small and Cl^- addition had minor effects on E_m.

Active Na^+ efflux

In order to keep Na^+ levels acceptable, plants growing at saline conditions must either have low passive Na^+ permeabilities or be able to maintain high efflux pumping capacity in the roots. When 50 mol m^{-3} NaCl and 20 mmol m^{-3} CCCP were added to the growth medium, after 6 hrs a dramatic increase in Na^+ influx could be detected especially in *P. media* (Fig. 2). Elevated Na^+ concentrations were accompanied by reduced K^+ concentrations, especially in the root tissue (Fig. 2a).

Discussion

From earlier studies it became clear that *P. maritima* and *P. media* differ in strategies toward high salinity: at low external NaCl *P. media* behaves like a sodium excluder, while *P. maritima* accumulates Na^+ ions (Table 1. De Boer, 1985; Erdei and Kuiper, 1979; Tanczos *et al.*, 1980).

However when NaCl concentrations increased (> 10 mol m^{-3}) *P. media* takes up more sodium than *P. maritima* (Table 1. De Boer, 1985). Both K^+/Na^+ uptake selectivity and K^+/Na^+ transport selectivity ratio (TSR) were higher in *P. media* than *P. maritima* under low or non salinity conditions (De Boer, 1985; Erdei and Kuiper, 1979). (TSR is defined as: $([K^+]_{shoot}/[K^+]_{root})/([Na^+]_{shoot}/[Na^+]_{root})$ Pitman, 1976; Stelter and Jeschke, 1983)). This conclusion can also be drawn from Table 1: when the external NaCl is increased, the TRS in *P. media* decreased, being 1 at about 10 mol m^{-3} NaCl (De Boer, 1985) and 0.19 at

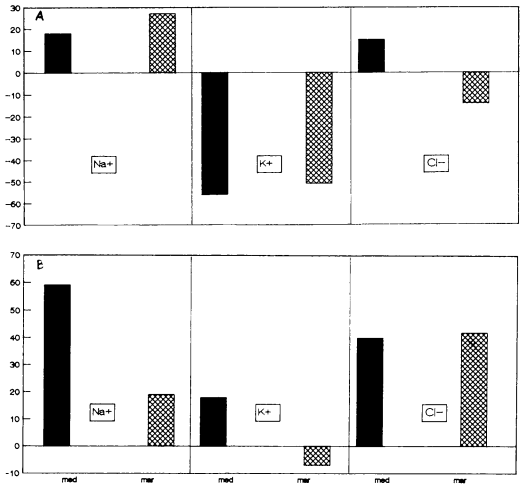

Fig. 2. Relative change (%) of K^+, Na^+ and Cl^- concentrations in roots (**A**) and leaves (**B**) of *P. maritima* (⊠) and *P. media* (■) due to CCCP in the growth medium.

25 mol m^{-3} (Table 1). TSR for *P. maritima* was 0.31 under these conditions.

Because comparable ionic strength resulted in less depolarization of the E_m in *P. maritima* (Fig. 1), overall conductance of the membrane must be less in this species. Also the almost similar values of E_m and K^+ Nernst potential in *P. media* reflects a high permeability for K^+ in this species. Lower K^+ and Na^+ permeability in *P. maritima* may be an advantage in salt tolerance since high influxes may be better controlled.

When electrogenic transport was inhibited by CCCP E_m depolarized approximately 40 mV (results not shown) and was determined by E_d and G_d (Eq. 1), E_d being the diffusion potential. This shows that also in *P. media* there still is a large electrogenic component in E_m in spite of the fact that E_m and the K^+ Nernst potential had nearly the same value. When E_d is measured and the ion activities are known (Table 1) relative permeabilities (α and β) can be calculated (Table 2). Under non saline conditions α was 0.58 in *P. maritima* which means the K^+/Na^+ selectivity ratio is 1.7.

On the other hand Figure 1 shows that under active conditions the depolarization caused by K^+ is about 3.5 times that caused by the same amount of Na^+. Apparently K^+/Na^+ uptake selectivity is more than halved, going from active to passive conditions. Assuming passive permeabilities for K^+, Na^+ and Cl^- to remain unaffected when

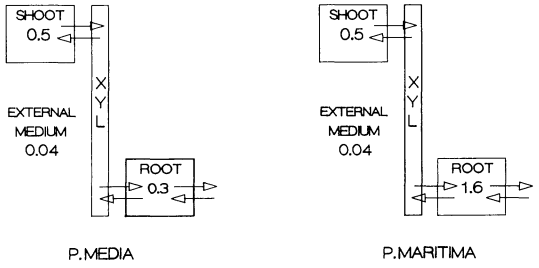

Fig. 3. K^+/Na^+ concentration ratios of external medium, shoot and root tissue in *P. maritima* and *P. media*. Plants were grown on 25% Hoagland and 25 mol m^{-3} NaCl. Shoot to root is appr. 1.6 for *P. maritima* and 3.0 for *P. media*.

CCCP is used, the decrease of K^+/Na^+ uptake selectivity can only be explained in terms of an increased Na^+ permeability under passive conditions. This in turn is caused by the inhibition of electrogenic H^+ transport responsible for driving H^+/Na^+ antiport (Fig. 4). For *P. media* at first sight this is less clear, since K^+/Na^+ uptake selectivity was relatively unaffected by CCCP, 3.5 and 4.0. Yet the plant is able to maintain a low Na^+_{root} concentration and a K^+/Na^+ ratio quite different from that offered in the growth solution. The K^+/Na^+ concentration ratio in the root cells was about 0.3 (Fig. 3) which is 7.5 times the ratio in the medium, while only a ratio of approximately 4 could be expected based on passive permeabilities (Table 2). For the plant as a whole ($K^+/Na^+ = 0.5$) this deviation was even more significant. With K^+ not far away from electrochemical equilibrium one expects active Na^+ efflux. When Na^+ efflux was inhibited by adding CCCP to the growth medium (25% Hoagland with 50 mol m^{-3} NaCl, Fig. 2), Na^+ levels rose dramatically: 50% on a whole plant basis in *P. media* and 22% in *P. maritima*. All these results indicate an active Na^+ efflux system in both *P. media* and *P. maritima* (Kylin, 1966; Tanczos *et al.*, 1980).

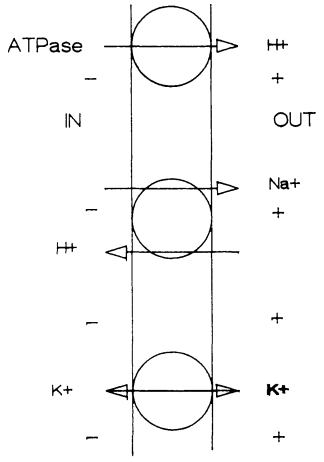

Fig. 4. Possible mechanisms for active Na^+ efflux pumping, situated on root cortex plasmalemma, driven by electrogenic proton pumps.

Surprisingly both species still kept their Na^+ root concentration low in the presence of CCCP, probably resulting from inaccessability of the xylem pumps for externally added CCCP (De Boer, 1985). This means that the major part of the above mentioned rise in Na^+ influx is caused by phenomena on the cortex medium boundary and that regulation on the symplast xylem boundary plus possible Na^+ retention in basal root parts may remain unaffected (Johanson and Cheeseman, 1983; Kramer, 1983; Nassery and Jones, 1976; Stelter and Jeschke, 1983; Walker, 1986).

Since no Na^+ ATPase is known in plant cells and K^+ is too close to equilibrium we suggest (Fig. 4) that the efflux pumping is based on a Na^+/H^+ antiport system driven by electrogenic ATPases. At these moderate salinities all transport is likely to be symplasmic (Moon *et al.*, 1986; Yeo and Flowers, 1986; Yeo *et al.*, 1987) and a Na^+ efflux pump at the cortex medium boundary may be a strong regulatory mechanism to prevent Na^+ levels from rising too much in the root cells, especially during the night when respiration is low and transport through the xylem may consequently be reduced.

Concluding, it seems *P. maritima* and *P. media* posses comparable Na^+ efflux mechanisms in the root cortex plasmalemma which only quantatively differ from each other. Apparently, regulation of this mechanism in *P. maritima* is such that Na^+ influx at higher external concentrations—up to 100 mol m^{-3}—can still be coped with while in *P. media* regulation ceases to function properly at these concentrations, resulting in strongly reduced growth of the plant.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 243–251.
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PLSO PN 160

Genotypic differences in boron accumulation in barley: Relative susceptibilities to boron deficiency and toxicity

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Key words: barley, boron, deficiency, tolerance, toxicity

Abstract

Boron accumulation in genotypes of barley displaying a large range of susceptibility to B toxicity was studied in solution culture experiments. The relationship between susceptibility to B toxicity and to B deficiency was also examined.

Plants were cultured for 5–6 weeks at B concentrations ranging from deficient to toxic and then examined for dry matter production and B contents of roots and shoots. The effect on grain production of B supply from soil was also studied.

At low and excessive B supply, respectively, symptoms of B deficiency and toxicity developed and growth was depressed. The extent to which individual genotypes were affected varied greatly and the responses closely reflected differences in B content.

At high B supply, genotypes with most tolerance to excess B accumulated less B in both roots and shoots than did genotypes susceptible to B toxicity. B concentrations in roots were always much lower than in shoots. No genotype displayed an ability to tolerate shoot B concentrations greater than 150–200 mg kg^{-1} dry matter without growth being depressed. It was concluded that tolerance to excess B in barley was governed by the ability of genotypes to restrict B uptake.

At low B supply, genotypes tolerant to excess B had relatively low tissue B concentrations compared to other genotypes, and were susceptible to B deficiency. Conversely, genotypes that had relatively high tissue B concentrations, and were susceptible to B toxicity, were the least susceptible to B deficiency.

Barley genotypes displayed marked differences in rates of B uptake (B accumulation), and these differences were independent of B supply. The order of genotypes when ranked for B concentrations in either roots or shoots was identical at all levels of supply. Similar patterns emerged with respect to grain production.

These results have two important implications. Firstly, they suggest that genotypes can be assessed simultaneously for relative susceptibility to both B deficiency and toxicity without imposing severe nutrient stress, because relative B concentrations of roots and shoots directly reflect these susceptibilities. Secondly, they point to a major complication for breeding programs aimed at incorporating either increased B efficiency or tolerance into commercial varieties.

Introduction

Boron toxicity is now recognized to occur widely and probably extensively in the cereal growing regions of South Australia (Cartwright *et al.*, 1986; 1987) and also Western Australia (M Riley, pers comm). Reduced grain production resulting from B toxicity is potentially a major problem. Since the removal excess boron from the soil is impractical in southern Australia, it has been necessary to seek a solution to the problem through the development of cereal varieties that tolerate B toxicity (Rathjen *et al.*, 1987). Several thousand barley and wheat genotypes have been screened and considerable

genotypic variation exists in tolerance to B toxicity. Thus the genetic basis exists for improving the tolerance to B toxicity in commercial varieties. The present experiments were undertaken as part of a study into the physiological basis of B tolerance (see also Nable, 1988). We show here that barley genotypes vary greatly in the extent to which B is accumulated, and this governs not only their susceptibility to B toxicity but also to B deficiency. Genotypes will differ greatly with respect to the soil B concentration at which yield is depressed by deficiency or toxicity, hence information is needed on soil and tissue concentrations of B which define the limits of normal growth among a wide range of genotypes. This information will assist in establishing critical values for B status for diagnostic application.

Materials and methods

Experiment 1

Five barley (*Hordeum vulgare* L.) genotypes, representing a range of susceptibility to B toxicity were cultured in nutrient solutions: Sahara 3763, Sahara 3769, Sahara 3768, Galleon and Schooner. Seeds were surface sterilized for 10 min in Na-hypochlorite (0.5% Cl), rinsed thoroughly, then soaked in deionized water for 2 d. Following imbibition, seeds were planted on plastic mesh in cups (6 seeds per cup) on lids over 5 L buckets containing aerated nutrient solution maintained at 15°C and pH 5.5–6.0.

The composition of the solution was (μM): KNO_3, 5000; $Ca(NO_3)_2$, 2500; $MgSO_4$, 1000; $NH_4H_2PO_4$, 500; FeEDTA, 100; NaCl, 10; $ZnSO_4$, 2.5; $CuSO_4$, 0.5; $MnSO_4$, 1; $CoSO_4$, 0.2; Na_2MoO_4, 0.1; H_3BO_3, 15. After 4 d the following B treatments were imposed in triplicate: 15, 200, 500, 1000, 2000 and 5000 μM H_3BO_3; these treatments will be referred to as B0.15, B2, B5, B10, B20 and B50, respectively which approximates to ppm ($\mu g\,L^{-1}$) and thus facilitates comparison between solution and soil culture. Nutrient solutions were replaced at 7 d intervals.

Plants were harvested 35 d after planting. Roots were washed for 30 min in 5 L 0.5 mM $CaSO_4$ (4°C), then rinsed for 1 min in deionized water to remove B and other nutrients from the free space (Bowen and Nissen, 1976). The plants were separated into roots and shoots, oven dried at 80°C, weighed, digested in acid and analysed for B by inductively coupled plasma spectrometry (Zarcinas and Cartwright, 1983).

Experiment 2

Three barley genotypes (Schooner, WI276, Sahara 3771) were grown in solution culture essentially as described for Experiment 1. However, to induce B deficiency, B contamination was removed from the nutrient solution by initially growing plants for 2 d in solutions to which was added approximately 6 g Amberlite IRA-743 B-specific anion exchange resin. The resin was then removed and the following B treatments imposed in triplicate: 0, 2.5, 7.5 and 15 μM H_3BO_3 (B0, B0.025, B0.075 and B0.15, respectively).

Plants were harvested after a further 24 d and at this time roots were washed, plants were separated into roots and remainder of the shoots, and analysed for B as described above.

Experiment 3

Three barley genotypes (WI-2723, WI-276, Sahara 3771) were cultured in a glasshouse experiment of 6 replicates using a bulk sample of topsoil (0–10 cm) of a red brown earth (J G Paull pers. comm.) from the CSIRO Glenthorne Research Farm, O'Halloran Hill, South Australia. The plants were grown in non-draining pots containing 1.7 kg soil to which H_3BO_3 was added to produce eight B treatments: 0, 10, 20, 30, 40, 60, 80, 100 mg B applied per kg soil (B0, B10, B20, B30, B40, B50, B60, B80 and B100, respectively). At the completion of the experiment mannitol extractable B levels were determined in the soil from each pot (Cartwright *et al.*, 1983):

B applied ($mg\,kg^{-1}$ soil)	0	10	20	30	40	60	80	100
Extractable B ($mg\,kg^{-1}$ soil)	0.3	5.4	8.8	14.2	20.0	34.1	41.9	47.4

Pots were watered regularly and brought to 70 percent of field capacity (field capacity approximately 38% w/w) at least once per week.

Seeds were germinated in darkness on damp filter paper for 4 d, and three seedlings were planted per pot. A basal fertilizer solution (25 mL per pot, containing 7.05 mg N, 2.03 mg P, 2.28 mg K, 7.06 mg S) was applied 24 and 44 d after planting out. At mid tillering (43 d after planting out), symptoms and tiller numbers recorded, and the shoots of plants from four replicates were harvested. Samples were dried at 80°C in a forced-draught oven, ground in a stainless-steel mill to pass a 1-mm screen and analysed for B as described above. The remaining two replicates were harvested at maturity and separated into grain and vegetative parts.

Results

Experiment 1

A square root transformation was used to normalize the growth data before analysis of variance was calculated. For ease of interpretation, the untransformed data are shown in Table 1. Statistical assessment however was based upon the transformed data.

At the control B supply of 15 μM H_3BO_3, plants grew well (Table 1) and developed no symptoms. As B supply increased, plants developed marked foliar symptoms of B toxicity and growth was depressed. The foliar symptoms of B toxicity were as previously described (Cartwright *et al.*, 1984); brown spots developed firstly at the tips and along the margins and later over most of the leaf blades. Older leaves were affected first.

The extent of symptom development differed greatly between genotypes for a given B supply. Thus, Schooner developed symptoms at B10 to B50 after only 6 d to 9 d and ultimately also at B2 and B5. By contrast, Sahara 3763 showed little symptom development even at B50.

The genotypes also displayed marked differences in the extent to which an excess B supply depressed growth (Table 1), with both roots and shoots showing essentially the same pattern. For example, the growth of Sahara 3763 was only significantly depressed at B50 ($P < 0.05$) and only by approximately 10 percent. On the other hand, Schooner had significantly depressed growth at a 10-fold lower B supply, B5 ($P < 0.001$).

The order of genotypes when ranked for susceptibility to B toxicity was identical for either foliar symptoms or growth, being Schooner, Galleon, Sahara 3768, Sahara 3769, Sahara 3763 (Table 1).

The internal B concentrations in roots and shoots of all genotypes increased with B supply (Table 2). Toxicity symptoms developed in shoots when their B concentrations were in excess of 80 mg kg^{-1} dry matter, which is consistent with a previous report for spring barley (Kluge and Podlesak, 1985).

As observed by Eaton (1944), internal shoot B concentrations were always greater than in roots; at B0.15 and B2 the difference was approximately

Table 1. Effect of an excessive B supply on dry matter production (mg per plant) of several barley genotypes (Experiment 1). Significance levels expressed relative to 15 μM B treatment and calculated from transformed data (square root transformation)

Genotype	Plant part	Treatment (μM)					
		15	200	500	1000	2000	5000
Sahara 3763	Roots	64.9	64.1	63.6	62.2	56.3*	60.3
Sahara 3769		43.9	47.4	44.3	42.1	42.7	48.5*
Sahara 3768		56.4	53.6	53.7	50.1*	49.8*	47.9*
Galleon		56.9	57.9	58.2	37.6*	33.9*	24.9*
Schooner		55.0	54.3	54.6	42.8*	19.2*	12.0*
Sahara 3763	Shoots	231.1	249.6*	240.6	236.6	240.9	218.0*
Sahara 3769		197.1	187.3	190.3	206.9	191.3	117.3*
Sahara 3768		169.8	161.6	164.2	164.6	136.1*	87.2*
Galleon		152.2	148.4	153.0	135.6*	127.7*	70.1*
Schooner		252.7	239.8	194.0*	177.6*	60.8*	35.8*

Values represent means of 3 replicates.
* — significant at 0.05 level.

Table 2. Effect of an excessive B supply on B concentrations (mg kg^{-1} dry matter) of several barley genotypes (Experiment 1)

Genotype	Plant part	B supply (μM)					
		15	200	500	1000	2000	5000
Sahara 3763	Roots	3 (1)	4 (2)	8 (2)	22 (1)	36 (5)	56 (4)
Sahara 3769		4 (1)	10 (2)	19 (2)	30 (5)	44 (5)	80 (15)
Sahara 3768		11 (1)	18 (8)	24 (6)	32 (3)	46 (3)	84 (2)
Galleon		14 (4)	27 (3)	36 (4)	51 (11)	80 (21)	154 (37)
Schooner		21 (3)	37 (3)	48 (4)	77 (5)	153 (13)	243 (20)
Sahara 3763	Shoots	8 (1)	12 (1)	19 (2)	40 (7)	65 (8)	130 (15)
Sahara 3769		11 (5)	44 (5)	71 (4)	118 (7)	180 (18)	246 (11)
Sahara 3768		21 (1)	69 (6)	109 (5)	159 (7)	261 (10)	457 (17)
Galleon		31 (4)	84 (9)	135 (8)	255 (32)	407 (34)	631 (44)
Schooner		37 (2)	91 (4)	152 (4)	238 (13)	350 (44)	1073 (114)

Values represent means of 3 replicates with S.D. in parentheses.

2-fold and up to 10-fold at B50. Roots of plants showing symptoms ranging from mild to severe contained from 23 to 331 mg B kg^{-1} dry matter, respectively, while shoots contained from 91 to 1226 mg B kg^{-1} dry matter, respectively. There was no indication that roots retained B, so preventing toxic accumulations in the shoots.

There were considerable differences between genotypes in their internal B concentrations for a given B supply (Table 2). The results indicate clearly that tolerance to an excess B supply was related to lower internal B concentrations. But even at B0.15, which was assumed to be a normal B supply, genotypes showed considerable differences in internal B concentrations. In fact at all levels of B supply, the genotype most susceptible to B toxicity — Schooner — had B concentrations in roots and shoots from 4- to 10-fold greater than in the least susceptible genotypes — Sahara 3763 and Sahara 3769. Moderately tolerant genotypes (*e.g.* Sahara 3769, Galleon) had intermediate concentrations. The ranking of genotypes for increasing internal B concentrations was identical with the order for increasing susceptibility to B toxicity. It was notable that these rankings were consistent over the range B0.15 to B50.

The amount of B in roots and shoots of all genotypes increased with B supply (Fig. 1), except at B50 (and also at B20 for Schooner) where B contents decreased, despite higher B concentrations (Table 2). This could be accounted for by the marked growth depression at the highest B treatment (Table 1).

The higher concentrations of B in shoots than roots was clearly reflected in the relative distribution of B between these organs (Fig. 1). As B supply increased, roots contained from 6 to 15 percent of the total B at B0.15 to 2 to 11 percent at B50. Again, there was no indication that B was being accumulated by the roots.

As with tissue B concentrations, genotypes susceptible to B toxicity contained appreciably more total B than tolerant genotypes, even at B0.15 (Fig. 1), despite there being no indication of B stress.

Experiment 2

Foliar symptoms of B deficiency only developed in the younger leaves of Sahara 3771 plants cultured at B0 and resembled those described by Robson and Snowball (1986); leaf blades were wrinkled, mishapen, irregularly chlorotic and occasionally had split margins (*i.e.* 'saw-tooth' edges) and longitudinal splits.

Root and shoot growth of Schooner (the genotype most susceptible to B toxicity) were unaffected by B supply, whereas both WI276 and Sahara 3771 showed reduced growth at B0, and B0 and B0.025, respectively (Table 3). The genotype least susceptible to B toxicity (Sahara 3771) was most susceptible to B deficiency, with both dry matter of roots and shoots being affected similarly. The B concentrations of roots and shoots clearly reflected these growth effects, with Sahara 3771 having the lowest concentrations and Schooner the highest at each level of B supply. Although no

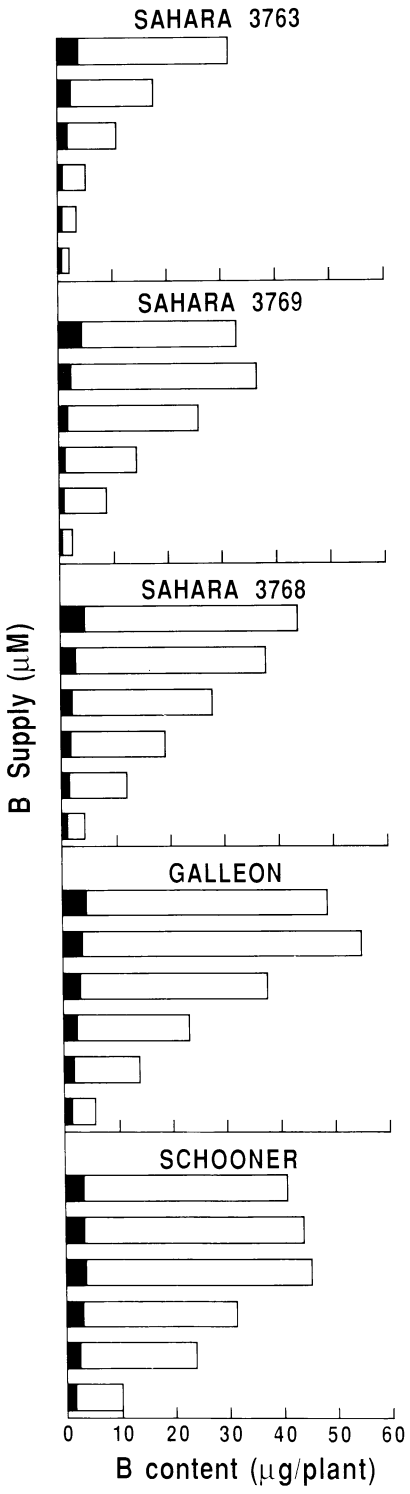

Fig. 1. The effect of B supply on the B content of several barley genotypes. Shaded and unshaded areas refer to roots and shoots, respectively.

tissue critical values are currently available for Australian barley varieties, shoot B concentrations less than approximately 5 mg kg^{-1} dry matter have been associated with B deficiency and reduced growth (Reuter, 1986), which is consistent with our results.

Experiment 3

When harvested at mid-tillering, both WI2723 and WI276 had developed typical foliar symptoms of B toxicity (Cartwright *et al.*, 1984); in both genotypes, these were mild at B20 and intensified at higher levels of B supply. By contrast, no foliar symptoms developed in Sahara 3771 at any level of B supply.

As expected, shoot growth was markedly decreased by excessive levels of B supply and contributing to this was a marked reduction in tiller production (Table 4). Growth decreased firstly between B10 and B20, B20 and B30, and B40 and B60 for WI2723, WI276 and Sahara 3771, respectively. Differences between genotypes in susceptibility to B toxicity were most obvious at the highest levels of B supply; whereas Sahara 3771 attained 50 and 35 percent of maximum growth at B80 and B100, respectively, the other genotypes showed almost no growth at all at these levels.

While these detrimental effects of excessive B supply were expected, it was observed that a positive response to B supply also occurred. Thus with the addition of B10 to the soil, growth (including tiller production) increased to a maximum above that observed without any B addition (Table 4). All genotypes showed this response, but it was most pronounced for Sahara 3771.

The B concentrations in the shoots of plants at mid-tillering again reflected the tolerance of each genotype to B toxicity (Table 4), with the susceptible genotype WI2723 accumulating considerably higher concentrations of B than the tolerant genotype Sahara 3771. These data suggest that shoot growth is only reduced when shoot B concentrations are in excess of 120 mg kg^{-1} shoot dry matter, the critical value determined by Kluge and Podlesak (1985) for barley. On the other hand, despite increases in growth from B0 to B10, at B10 all genotypes when grown without additional B had greater than 10 mg B kg^{-1} shoot dry matter, and so would not normally be considered B deficient.

Table 3. Effect of low B supply on growth and B concentrations of three barley genotypes (Experiment 2)

Genotype	Plant part	B supply (μM)			
		0	2.5	7.5	15
Dry matter (mg per plant)					
Schooner	Shoots	129 (9)	140 (9)	132 (28)	121 (16)
WI 276		68 (5)	86 (3)	92 (8)	99 (12)
Sahara 3771		74 (1)	84 (11)	92 (19)	107 (7)
Schooner	Roots	38 (2)	40 (7)	44 (7)	37 (5)
WI 276		23 (2)	40 (3)	37 (1)	36 (2)
Sahara 3771		17 (3)	35 (4)	33 (4)	37 (5)
B concentration (mg kg^{-1} dry matter)					
Schooner	Shoots	5.6 (0.9)	10.0 (0.6)	22.1 (4.0)	46.4 (9.4)
WI 276		3.7 (0.4)	9.0 (1.6)	19.4 (3.1)	29.4 (5.3)
Sahara 3771		2.5 (0.6)	5.5 (0.3)	7.8 (0.5)	11.7 (1.6)
Schooner	Roots	N.D.	2.6 (0.4)	4.5 (0.1)	5.8 (0.3)
WI 276		N.D.	2.5 (0.3)	4.8 (1.0)	7.0 (1.9)
Sahara 3771		N.D.	1.6 (0.1)	3.1 (0.6)	3.1 (0.7)

N.D. — below detection limits.
Values represent mean of 3 replicates with s.e. in parentheses.

At maturity, the pattern of response of shoot growth to B supply (Table 5) was the same as the first harvest; all genotypes produced more shoot DM when grown with the addition of B10 than without any B addition, and as B supply increased to toxic levels, shoot growth decreased. The relative responses of genotypes was also identical. The effect of excessive B supplies (*i.e.* > 10 mg kg^{-1} soil) on shoot growth was reflected in grain production, with grain number per plant, grain number per head and 1000-grain weight all diminishing as B supply increased (Table 5). This effect was most pronounced in WI2723, where grain number per plant was 38 percent lower at B20 than at B10 and no grain was produced at B80 and B100, whereas in Sahara 3771 production decreased by only 16 percent at B40, and even at B100 plants still produced 35 percent of maximum yield. Sahara 3771 plants grown at B concentrations ranging from 10 to 100 mg kg^{-1} soil produced the same number of

Table 4. The effect of B supply on shoot growth, tiller production and B concentrations in three barley genotypes harvested at mid-tillering (Experiment 3)

Genotype	B applied (mg kg^{-1} soil)							
	0	10	20	30	40	60	80	100
Shoot dry matter — excluding grain (g per plant)								
WI2723	1.04 (0.06)	1.27 (0.05)	0.62 (0.07)	0.78 (0.06)	0.17 (0.02)	0.20 (0.03)	0.07 (0.02)	0.05 (0.01)
WI276	1.09 (0.07)	1.33 (0.01)	1.27 (0.03)	1.03 (0.06)	0.29 (0.02)	0.27 (0.03)	0.08 (0.02)	0.04 (0.01)
Sahara 3771	0.89 (0.05)	1.26 (0.09)	1.25 (0.06)	1.31 (0.6)	1.20 (0.5)	1.05 (0.06)	0.62 (0.04)	0.39 (0.3)
Tiller number per plant								
WI2723	9.8 (0.7)	10.9 (0.5)	6.1 (0.7)	6.2 (0.4)	3.2 (0.4)	3.3 (0.6)	1.0 (0.1)	1.0 (0.1)
WI276	9.6 (0.7)	11.2 (0.4)	7.9 (0.5)	7.7 (0.8)	3.8 (0.4)	3.8 (0.3)	1.3 (0.1)	1.0 (0.1)
Sahara 3771	7.1 (0.4)	10.3 (0.8)	9.6 (0.4)	9.6 (0.6)	7.6 (0.4)	7.4 (0.6)	3.8 (0.3)	3.4 (0.4)
Shoot B concentration (mg kg^{-1} dry matter)								
WI2723	19 (3)	192 (16)	588 (86)	877 (139)	1563 (98)	1976 (181)	*	*
WI276	15 (2)	154 (11)	352 (43)	461 (87)	878 (121)	1310 (101)	*	*
Sahara 3771	11 (2)	70 (12)	120 (19)	230 (54)	387 (101)	489 (67)	*	*

Values represent means of four replicates with s.e. in parentheses.
* — data unavailable.

Table 5. The effect of B supply on shoot growth and grain production in three barley genotypes harvested at maturity (Experiment 3)

Genotype	B applied (mg kg^{-1} soil)							
	0	10	20	30	40	60	80	100
Shoot dry matter—excluding grain (g per plant)								
WI2723	6.4	8.4	8.4	7.5	6.5	2.8	0.3	0.3
WI276	6.6	10.9	11.5	10.8	9.1	8.6	1.3	0.8
Sahara 3771	4.7	6.1	6.4	6.8	6.5	5.9	5.0	3.8
Grain number per plant								
WI2723	108	84	52	50	18	6.5	0	0
WI276	132	74	58	42	7.5	1.2	0	0
Sahara 3771	92	143	144	134	104	88	91	49
Grain number per head								
WI2723	9.6	8.3	8.0	8.3	5.5	5.4	0	0
WI276	17.6	11.4	9.2	9.3	3.3	1.0	0	0
Sahara 3771	19.6	25.1	25.3	24.4	23.1	23.8	24.0	24.5
1000 grain weight (g)								
WI2723	43	41	43	34	34	28	–	–
WI276	34	38	35	40	28	25	–	–
Sahara 3771	38	34	33	35	38	36	25	20

Values represent means of two replicates.

grains per head, whereas in the other genotypes grain number per head was markedly reduced at the higher levels of B supply.

While for all genotypes shoot growth increased with the addition from B0 to B10, grain production increased only for Sahara 3771 (Table 5). In the latter case, grain number per plant and grain number per head were increased by 55 and 28 percent, respectively. In WI2723 and WI276 grain number per plant decreased by 22 and 44 percent, respectively while grain number per head decreased by 14 and 35 percent respectively. However, growth response after anthesis to applied B in pots is often not a reliable guide to performance in the field (Paull *et al.*, 1990).

Discussion

The barley genotypes that were examined displayed different abilities to accumulate B which were independent of B supply, and reflected their respective susceptibilities to both B toxicity and B deficiency. When the results of Experiments 1 and 2 are considered together, they indicate that B accumulation is independent of external B supply. Thus, barley genotypes displayed differences in ability to accumulate B that were maintained at levels of B supply from deficient to toxic. For example, the genotype Sahara 3771 accumulated the least B at all levels of B supply and was the least susceptible to B toxicity and the most susceptible to B deficiency. This relationship between B accumulation and B nutrition in barley genotypes complements previous reports of differences between species. For example, Oertli and Roth (1969) showed that soybeans were more susceptible than sugar beet to B toxicity but less susceptible to B deficiency, with the differences being due to B accumulation. Similarly, Henkens (in Millikan, 1961) reported that crops which were more sensitive to B deficiency were least susceptible to B toxicity.

We stress at this point, that although there is an abundant literature on comparisons between species for susceptibility to B toxicity and deficiency (*e.g.* Bradford, 1971; Eaton, 1944; Gupta *et al.*, 1985), such comparisons are based upon the assumption that one or a few genotypes can typify a species. The present results show clearly that for barley, at least, this assumption is completely invalid. So we must now question whether any of the interspecific comparisons have any applicability beyond the genotypes actually tested. Moreover, the same query may apply equally to other mineral nutrients.

To our knowledge, this inverse relationship between susceptibility to deficiency and toxicity has

not been reported for other nutrients from which plants may suffer both extremes of nutrition (*e.g.* Mn, Cu, Zn). Ohki *et al.* (1980) found that soybean genotypes had similar rankings for susceptibility to both Mn deficiency and toxicity, in total contrast to the present results. Although Munns *et al.* (1963) reported that oat genotypes maintained differences in shoot Mn concentrations over a range of Mn supplies from 0.2 to 2000 μM, no data was presented on the relative susceptibilities of the genotypes to Mn deficiency and toxicity.

The mechanism which causes these genotypic differences in B accumulation is unknown, but presumably involves different rates of absorption, active exclusion or active efflux. While Raven (1980) claims that B absorption is predominantly a passive process, greatly influenced by transpiration rate, this may be relavant to only a very limited range of genotypes. In fact, Nable (1988) showed that differences in B accumulation amongst the genotypes investigated here could not be accounted for by differences in transpiration rates. Other factors that may differ between genotypes and substantially influence passive absorption of B include: a) surface area of the roots; b) composition of the root cell membranes and resulting effects on permeability to B; and c) concentration of B adsorption sites in the free space, in particular the cis-diol content (Nable, 1988). No information is available on the genetic variation for these characteristics. Likewise, information is unavailable to permit any speculation on possible differences between genotypes in regard to active mechanisms for uptake, exclusion or efflux of B.

The present results support the hypothesis of Rathjen *et al.* (1987), that a consequence of improvement in tolerance to toxicity without increased flexibility of adaptation must be an increase in susceptibility to deficiency. Consistent with this notion are observations that Janak wheat was more susceptible to B deficiency than Sonalika (Chatterjee *et al.*, 1980), whereas their susceptibilities to B toxicity in soil were in reverse order (Rathjen, unpublished data).

B nutrition is more problematical in this respect than other mineral nutrients from which plants may suffer either deficiency or toxicity for two reasons. Firstly, both the difference between deficient and toxic levels of B in plant tissues, and between deficient and toxic levels of B supply (as seen in the present experiments) are unusually narrow compared to the range for other nutrients (Eaton, 1944; Reisenauer *et al.*, 1973). Thus, small changes in the capacity to accumulate B can have marked effects on B status. Secondly, because both B deficient and toxic soils can sometimes occur in close proximity the choice of appropriate fertilizer practices and cereal varieties can be confounded. For example, the mallee lands of southern Australia contain sandy dunes with intervening swales of heavier texture. Boron-rich soils in these landscapes are confined to swales, while adjacent sand ridges may have low available B.

The next step is to examine genotypic variation in critical tissue levels for both B deficiency and toxicity. If such variation exists, it may be possible to recombine genetically the determinants of efficiency and tolerance within tissues. That is, to introduce either B efficiency (a lower critical tissue level for deficiency) into a genotype with restricted B uptake or B tolerance (a higher tissue critical level for toxicity) into a genotype with high rates of B uptake. In both cases, genotypes should then be adapted to a wider range of soil B concentrations.

Acknowledgements

The work was funded in part by a grant to RO Nable from the Australian Barley Research Council. We thank Ms L R Spouncer and Ms F C N Munn for excellent technical assistance, and Mr N Horne for establishing Experiment 3.

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PLSO PN 161

Behaviour of different wheat genotypes under various irrigation conditions in semi-arid tropics of Haryana, India

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Key words: irrigation conditions, saline water, semi-arid tropics, wheat genotypes

Abstract

Field experiments were conducted on sandy loam (Typic Ustochrepts) soil at Hisar, India in winter seasons of 1982–83 through 1985–86. Wheat genotypes *viz.*, WH-157, WH-283, WH-820 and VW-108 (1982–83 and 1983–84) and WH-157, WH-291, Kharachia 65 and HD-2009 (1984–85 and 1985–86) were tested under three irrigation conditions *viz.* dry (no post sowing irrigation), irrigation at irrigation depth/cumulative evaporation (ID/CPE 0.9), with normal and saline waters. Normal water irrigation produced significantly higher grain yield than dry and saline irrigations, per cent reductions being 28.69 and 47.07 (1982–83, 1983–84) and 15.67 and 30.38 (1984–85, 1985–86) with saline and dry irrigation conditions, respectively. Wheat genotypes VW-108 and WH-157 recorded high grain yields all through 1982–1986. Performance of Kharachia 65, however, did not vary under different irrigation conditions. Analysis on correlation and genetic advance indicated that effective tillers m per row length were positively correlated with number of spikelets per ear. Genetic advances for grain yield and ear length, in general, were low under all irrigation conditions and moderate for number of grains per ear and 1000 grain weight. Consumptive use of water by wheat was higher under normal water irrigation while water-use efficiency (WUE) was higher with dry irrigation condition. Genotypes VW-108 and WH-157 used consumptively highest and Kharachia 65 lowest water. Irrespective of genotypes, salt buildup (Ec dsm^{-1}) was maximum under saline water irrigation.

Introduction

Crop performance and yield are the result of genotypic expression as modulated by continuous interactions with the environment. Among the environmental factors, the water and its salinity are most widely limiting for crop production on a global basis in general and arid and semi-arid regions in particular. In arid and semi-arid areas where alternative irrigation facilities are not affordable or possible, if crops could be grown with brackish water, it would open up large resources of water for irrigation provided ways could be found out to use these waters for irrigation. About 67 per cent underground waters in semi-arid tropics of Haryana, India have been characterized as unsafe for irrigating crops (Chamola and Kumar, 1984; Singh Harbir *et al.* 1985) in accordance to the international standards (Richards, 1954). Despite of the warning by Richards (1983) that selection for improved salt tolerance crops/genotypes under field conditions may be difficult because of spatial variability inherent in saline soils/soils irrigated with saline water some progress has been made using controlled conditions (Downton, 1984; Epstein and Norlyan, 1977). Intensive studies were, therefore, undertaken at the crop research Centre of the Haryana Agricultural University, Hisar to compare the relative behaviour of some wheat genotypes under dry, normal and saline irrigation conditions.

Materials and methods

The investigations were carried out at the Crop Research Centre of the Haryana Agricultural University, Hisar — 125 004 (India) at an altitude of

215.2 m. MSL and at latitude and longitude of 29°10′ and 75°44′ N and E, respectively. The soil was sandy loam (typic ustechrepts) consisting 65.60, 16.20, 17.87 per cent, sand, silt and clay, respectively. The moisture content was 17.0 per cent at −0.03 MPa and 7.1 at −1.5 MPa. The bulk density of upper profile (120 cm) ranged from 1.41 to 1.48 g cm^{-3} and the pH from 7.7 to 8.1.

The experiment was laid out in split block design in three replications with twelve treatment combinations consisting of three irrigation conditions viz. dry (no-post sowing irrigation), irrigations at ID/CPE, 0.9 with normal water and saline water and four wheat (*Triticum aestivum* L.) genotypes *viz.*, WH-157, WH-283, WH-820 and VW-108 (1982–84) and WH-157, WH-291, HD-2009 and Kharachia 65 (1984–86).

After applying pre-sowing irrigation (normal water) wheat (*Triticum aestivum* L.) was sown as per genotype by drilling the seed in rows spaced 20 cm apart using 100 kg seed ha^{-1}. A basal dose of nitrogen and phosphorus at the rate of 120 kg N and 60 kg P_2O_5 ha^{-1} was applied every year in all treatments through urea and single super phosphate, respectively. A buffer channel of 0.75 m width was provided on all the four sides of each 5 × 3 m plot to avoid seepage effects.

In the treatments irrigation with normal and saline water, a measured quantity of irrigation water was applied whenever ID/CPE value reached 0.9. The water used in saline irrigation treatment contained 39.8, 3.3, 22.8, 6.0, 20.5, 21.0 and 18.7 meq l^{-1} of Na^+, Ca^{2+}, Mg^{2+}, CO_3^-, HCO_3^-, Cl^- and SO_4^{2-}, respectively and EC of 9.4 dsm^{-1} with pH 8.3. The active root zone (120 cm) was brought to field capacity with each irrigation.

Observations recorded were effective tillers per metre row length, ear length, spikelets per ear, grains per ear and 1000-grain weight at harvest. For grain yield the crop was harvested at maturity. The air dry weight of grain for 10.4 m^2 plot was recorded. This observation was used for computing grain yield q ha^{-1}. The mean of observations recorded at harvest were used for working out genotypic and phenotypic correlations and genetic advances of genotypes used in these investigations. Water-use efficiency was calculated as grain yield (q ha^{-1}) divided by total seasonal evapotranspiration (consumptive use), mm (ET = Water added + Effective rainfall + Δsoil). Due to low intensity of rainfall during all the years, the total amount of rainfall received was considered as effective rainfall. Soil salinity in terms of EC (dsm^{-1}) of 1:2 saturation extract was monitored before sowing and after harvesting in each crop season.

Results and discussion

Grain yield and yield attributes

The differences between genotypes, irrigation conditions, and the interaction between both (1985–86 only), were statistically significant (Table 1a, b). The distinct superiority of WH-157 and VW-108 in yielding capabilities is quite apparent. Kharachia 65 a tall indegenous salt resistant genotype gave lowest grain yield. However, WH-283, HD-2009 and WH-291 recorded substantially good yields. The interaction table indicating plant genotypes responses to irrigation differences as the irrigation conditions shows significant yield differences as the irrigation conditions changed commencing from dry (no post sowing irrigation) to the saline and normal irrigation. Significantly higher, number of effective tillers per metre row length, number of spikelets, grains per ear remained with WH-157 and VW-108 genotypes and this could be attributed to higher grain yields recorded with above genotypes (Table 2). The results reported by Singh and Malik (1983) and Singh *et al.* (1988) corroborate the observations recorded in the present study.

The association in terms of nature and magnitude of yield of genotypes is influenced by the environmental conditions as genotypic correlations were slightly higher than corresponding phenotypic ones which suggest that experimental errors were minimum and phenotype was good indicator of genotype of wheat. Number of effective tillers per metre row length in dry and saline irrigation conditions were positively associated with spikelets and grains per ear. Like that ear length exhibited positive association with grain yield under normal water irrigation whereas spikelets per ear and grains per ear showed positive correlations with yield under normal and saline water irrigations (Table 3).

In general, genetic advances for grain yield and ear length were low in all the irrigation conditions

Table 1a. Grain yield and water use efficiency (WUE) of wheat as influenced by irrigation conditions and genotypes

Treatments	Grain yield ($g\,ha^{-1}$)				WUE ($kg\,ha^{-1}\,mm^{-1}$)
	1982–83	1983–84	1984–85	1985–86	
Irrigation conditions					
Dry	24.72	23.72	32.72	33.09	12.15
Normal	47.08	44.45	46.36	48.18	11.26
Saline	32.27	33.00	40.00	39.72	11.32
L.S.D.	1.09	1.27	1.26	1.38	
Genotypes					
WH-157	35.99	34.90	–	–	12.45
WH-283	27.54	28.72	–	–	11.12
WH-820	28.54	29.09	–	–	11.22
VW-108	46.72	42.27	–	–	12.42
Kharachia 65	–	–	28.18	26.09	9.0
WH-157	–	–	48.18	47.27	12.5
WH-291	–	–	40.00	43.90	11.5
HD-2009	–	–	42.72	44.18	12.0
L.S.D.	1.33	1.57	2.36	1.92	

Table 1b. Grain yield of wheat as influenced by irrigation conditions × genotypes ($q\,ha^{-1}$)

Treatment	Dry	Normal	Saline
WH-157	37.27	58.90	45.54
WH-291	34.54	53.63	43.63
Kharachia 65	25.54	26.63	26.00
HD-2009	35.09	53.45	43.90
L.S.D.		2.38	

whereas these were moderate for grains per ear and 1000 grain weight. Genetic advance was, however, highest for effective tillers per metre row length (Table 4). Genetic advances per se were also influenced by the irrigation conditions and in general, higher in normal water irrigation for all the traits. Genotype WH-291 recorded the high genetic advance for effective tillers per metre row length.

Table 2. Grain yield components of wheat as influenced by different treatments

Treatments	Effective tillers per m row length		Ear length (cm)		Spikelets per spike		Grains per ear		1000-grain weight (g)	
	1982–84 (mean)	1984–86 (mean)	1982–84 (mean)	1984–86 (mean)	1982–84 (mean)	1984–86 (mean)	1982–84 (mean)	1984–86 (mean)	1982–84 (mean)	1984–86 (mean)
Irrigation conditions										
Dry	57.5	74.3	8.65	8.38	15.8	21.6	34.9	40.6	39.1	39.7
Normal	78.9	89.8	9.30	9.20	16.9	25.6	49.4	48.0	38.0	41.4
Saline	67.8	84.1	8.78	9.53	15.8	23.5	55.9	46.0	39.5	42.1
L.S.D.	2.45	3.3	NS	0.12	0.56	2.12	5.12	1.37	0.29	0.38
Genotypes										
WH-157	70.6	–	–	8.90	16.3	–	38.7	–	44.9	–
WH-283	64.4	–	–	9.42	15.2	–	36.2	–	44.1	–
WH-820	65.5	–	–	7.27	15.6	–	36.3	–	32.6	–
VW-108	79.7	–	–	9.84	16.8	–	38.2	–	34.0	–
Kharachia 65	–	95.5	9.27	–	–	23.7	–	45.5	–	38.0
WH-157	–	93.3	9.07	–	–	24.3	–	40.5	–	44.8
WH-291	–	73.8	7.98	–	–	22.9	–	44.1	–	43.6
HD-2009	–	68.4	9.50	–	–	24.0	–	49.5	–	37.8
L.S.D.	1.22	3.07	NS	0.24	0.30	NS	NS	0.55	0.38	0.58

Table 3. Genotypic and phenotypic correlation between grain yield and its components in wheat

Character			Effective tiller	Ear length	Spikelets per spike	Grains per ear	1000-grain weight	Grain yield
Effective tillers		1	1.0000	0.1809	0.8281	0.7622	−0.0489	−0.3884
		2	1.0000	−0.4328	0.3089	0.1134	0.2089	−0.3917
		3	1.0000	0.1698	0.5165	0.5293	−0.1142	−0.1646
Ear length	GENOTYPIC	1		1.0000	0.1970	0.0157	0.3152	0.1052
		2		1.0000	0.2751	0.5919	0.1287	0.6793
		3		1.0000	0.4006	0.4124	0.0823	0.5416
Spikelets per spike		1			1.0000	0.9998	−0.1725	0.5435
		2			1.0000	0.9914	0.2138	−0.0514
		3			1.0000	0.9993	0.1858	0.6992
Grains per ear		1				1.0000	−0.0841	0.5165
		2				1.0000	0.1347	0.1302
		3				1.0000	0.1381	0.6991
1000-grain weight		1					1.000	−0.1954
		2					1.0000	0.0287
		3					1.0000	0.1823
Effective tillers		1	1.0000	0.1337	0.8112	0.6512	−0.2101	0.2349
		2	1.0000	−0.3097	0.2771	0.0517	0.2089	−0.3777
		3	1.0000	0.1542	0.5122	0.4728	−0.1149	−0.1338
Ear length	PHENOTYPIC	1		1.0000	−0.0354	0.1881	0.1712	−0.0132
		2		1.0000	0.2244	0.3287	0.0765	0.4506
		3		1.0000	0.3067	0.4239	0.0367	0.3156
Spikelets per spike		1			1.0000	0.7811	−0.1647	0.4849
		2			1.0000	0.9087	0.2124	−0.0225
		3			1.0000	0.9124	0.1761	0.5310
Grains per ear		1				1.0000	−0.0712	0.4158
		2				1.0000	0.1154	0.1222
		3				1.0000	0.1202	0.5310
10000-grain weight		1					1.0000	−0.1889
		2					1.0000	0.0306
		3					1.0000	0.2192

1 = Dry, 2 = Normal, and 3 = Saline irrigation.

This may be explained on the basis that genotype WH-291, a short duration, early maturing, thus might have escaped the drought under dry and saline irrigation conditions. Genetic advance for grains per ear was highest with Kharachia 65 (tall, local, salt resistance genotype) in dry and saline irrigation conditions compared to normal one. In that analogy genotypes WH-157 and HD-2003 were good for 1000-grains weight. Crossing of the genotypes for recombing potential yield advance per se in stress tolerance is suggested (Table 3).

Water use and water use efficiency

The water use efficiency expressed as kg grain per hectare per mm evapotranspiration (Table 1a) was more with dry irrigation treatment and decreased with irrigation either with saline or normal water and the decrease was observed as the degree of water/salinity stress diminished. Higher water-use efficiency by pearlmillet under dry moisture regime has also been reported under moderate drought condition (Jarwal, 1985) and in wheat under saline irrigation condition (Singh *et al.*, 1988).

Root zone salinity

The estimated average root zone salinities (1:2 saturation extract) that have been resulted under different irrigation conditions are illustrated in Figure 1. The range in salinities is considerable depending on the irrigation condition(s). A com-

Table 4. Genetic advances of different componental characters in wheat

Genotype		Componental character	WH-157	WH-283	WH-820	VW-108	WH-291	HD-2009	Kharachia 65
A.	*Dry irrigation*	77.36118550							
1.	Effective tillers		58.37	53.73	55.73	62.33	89.90	62.00	64.00
2.	Ear length		9.03	8.83	7.63	9.10	8.73	7.03	8.70
3.	Spikelets spike^{-1}		15.93	15.47	15.07	16.87	21.93	19.00	20.17
4.	Grain ear^{-1}		36.27	34.67	33.33	35.33	42.50	38.30	43.23
5.	1000-grain weight		45.40	44.74	32.28	34.08	37.30	40.00	36.10
6.	Grain yield		3.18	2.01	2.15	3.98	3.03	3.77	3.70
B.	*Normal water irrigation*	76.96624376							
1.	Effective tillers		81.47	76.33	76.43	81.33	99.03	90.63	71.27
2.	Ear length		9.70	9.37	8.13	10.00	9.10	8.83	10.73
3.	Spikelets spike^{-1}		17.27	16.13	17.13	17.20	24.50	27.23	28.13
4.	Grains ear^{-1}		42.53	39.60	41.00	42.40	47.93	50.17	56.00
5.	1000-grain weight		43.80	43.29	31.80	33.35	38.03	44.70	37.93
6.	Grain yield		5.92	4.04	4.28	6.19	3.37	5.20	5.50
C.	*Saline water irrigation*	62.90289694							
1.	Effective tillers		72.07	63.03	64.03	71.63	97.77	68.87	69.93
2.	Ear length		9.10	9.03	7.68	9.43	8.93	8.07	10.10
3.	Spikelets spike^{-1}		15.80	16.20	14.80	16.30	22.83	21.67	23.93
4.	Grain ear^{-1}		37.47	34.40	34.67	37.07	45.97	44.00	49.33
5.	1000-grain weight		45.60	44.24	33.69	34.59	38.73	46.13	39.63
6.	Grain yield		4.07	3.25	3.09	4.53	3.37	5.20	5.50

parison of the estimated mean salinities under different irrigation conditions shows that the soil salinities in saline water irrigated plots were more than those of dry and normal water irrigated ones. Such a trend was not unexpected as saline water used in these studies had a higher load of soluble salts as compared to normal (canal) water.

Several favourable conclusions may be drawn from this study (i) wheat has marked genotypic and phenotypic plasticity enabling it to make appreciable physiological adjustments to dry/saline irrigation condition (ii) wheat seems to have quite higher genotypic diversity making it possible to select and recombine for dry/saline irrigation conditions (iii) selection for salt tolerance from many genotypes of wheat, followed by a breeding programme can confidently be expected to increase the yields under dry/saline irrigation conditions in near future.

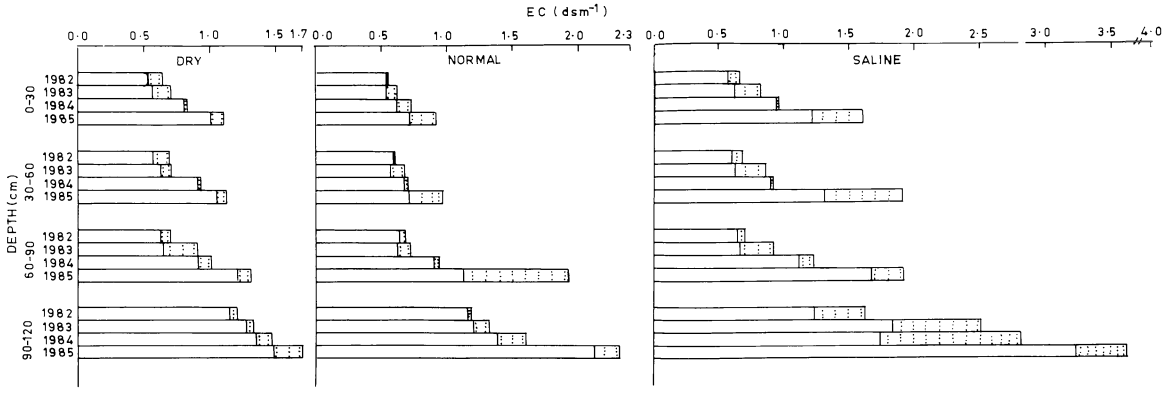

Fig. 1. Electrical conductivity (dsm^{-1}) of 1:2 saturation extract of soil under wheat with different irrigation conditions. The *shaded bars* indicate the increase in EC of soil after crop growing season.

Acknowledgements

The junior author is thankful to the Alexander von Humboldt foundation, Jean-Paul-Str. 12, Bonn-2, FRG for providing financial assistance for the presentation of the paper in Third International Symposium on Genetic Aspects of Plant Mineral Nutrition at Braunschweig (FRG).

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 259–262.
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PLSO PN 162

Iron tolerance of rice cultivars

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Key words: flooded rice, iron toxicity, nutrient uptake, root and shoot dry weight

Abstract

Development of cultivars of flooded rice, which are tolerant of iron toxicity is an important step in reducing production costs and increasing rice yields in many parts of the world. Success has been limited by inadequate screening techniques and the lack of sufficient data showing clear differences in response to well defined iron stress. Twelve flooded rice (*Oryza sativa* L.) cultivars were evaluated over three iron concentrations (90, 890, and 1780 μM Fe) in nutrient solution. Based on shoot dry weight, cultivars were classified relative to tolerance of iron toxicity. Rice cultivars differ in their response to iron toxicity. The tolerant cultivars at the highest Fe concentration were P3083F4-61, CNA 1051, CNA 3814, and P3304F4-27. High Fe concentration in the growth medium reduced uptake of P, K, Zn and Mn.

Introduction

Irrigated or flooded rice is grown on flat land with controlled irrigation. The soil is commonly flooded to 10 to 15 cm when seedlings attain 25 to 30 days, and flooding is maintained up to a week or 10 days before harvesting.

Due to flooding, chemical properties of flooded rice soils are entirely different from those of drained soils. The reduced soil conditions cause some changes which are beneficial as well as harmful. One harmful effect is increased concentration of Fe^{2+} which can reach toxic levels for the rice crop. Plants generally show bronzing if soluble iron in the rooting medium is in the 300 to 500 mg kg^{-1} range (Breeman and Moorman, 1977). The symptoms of iron toxicity are characterized by the development of scattered small reddish brown spots on the lower leaves. In severe cases, the whole leaf becomes brown and the lower leaves turn dark grey and die. These symptoms are sometimes similar to P deficiency.

Iron toxicity in flooded rice has been reported in Southeast Asia, Africa, and South America (Fageria and Rabelo, 1987). Iron toxicity may be attributed to high iron content in the soil, low soil pH, low soil fertility, and/or accumulation of harmful organic acids and/or hydrogen sulfide (Inda, 1966; Ota and Yamada, 1962; Tanaka *et al.*, 1966; Trolldenier, 1973).

The objective of this study was to test the reaction of several rice cultivars to iron concentrations in nutrient solution. Promising materials might then be used in field trials or in breeding programs.

Materials and methods

A solution culture experiment was conducted to study the influence of iron on growth and uptake of nutrients by 12 rice cultivars. With slight modifications, the solution was based on that recommended by the International Rice Research Institute in the Philippines for rice. The nutrient solution had the following composition in μM: NH_4NO_3, 2857; $NaH_2PO_4\,H_2O$, 129; K_2SO_4, 1030; $CaCl_2$, 1000; $MgSO_4\,7H_2O$, 1640; $(NH_4)Mo_7O_{24}\,4H_2O$, 0.5; $MnCl_2\,4H_2O$, 9; H_3BO_3, 18.5; $ZnSO_4\,5H_2O$, 0.15; and $CuSO_4$, 0.16. Iron was supplied as FeEDTA in the amount required for Fe concentra-

tions of 90, 890 and 1780 μM. These Fe levels were optimum, medium and highly toxic based on an earlier work (Fageria *et al.*, 1984). Nutrient solutions were changed weekly. The pH of the solution was adjusted to 4 $\pm$ 0.2 initially and once every two days thereafter with 0.1 M NaOH or 0.1 M HCl. The experiment was conducted in a randomized block design with two replications. Maximum and minimum air temperatures during the experiment were 28 $\pm$ 2°C and 18 $\pm$ 2°C, respectively.

Seeds of 12 rice cultivars were germinated in nutrient solution using 2-liter plastic pots. When seedlings attained an age of 18 days, 4 uniform seedlings of each cultivar were transplanted to acrylic discs with holes in the center and held in place with non-absorbent cotton. Discs were then transferred to nonaerated plastic pots containing about 8 liters of nutrient solution with the different iron treatments.

After 35 days growth in Fe-treated solutions, plant shoots and roots were harvested separately and washed in distilled water. Plant material was dried to a constant weight at about 70°C and dry matter was determined. Ground material was digested with a 2:1 mixture of nitric and perchloric acid. The P concentration in the digest was determined colorimetrically, while all other elements were determined by atomic absorption spectroscopy. Total N in the tissue was determined using a Tecator 1016 digester and 1004 distilling unit.

Statistical Analysis System (SAS) programs were used for analysis of variance and to calculate correlation coefficients and regression equations relating growth and nutrients uptake parameters.

Results and discussion

The activity of FeEDTA in the nutrient solution at different pH and iron concentrations was calculated using the GEOCHEM computer program (Sposito and Mattigod, 1980). The calculated activity of FeEDTA was nearly constant from pH 4 to 7 in each of the three Fe concentrations (Table 1), indicating that FeEDTA should have provided a stable iron supply for the plants.

Analysis of variance was used to examine the main effects of cultivars (CV), Fe levels and interactions of CV $\times$ Fe. F-values for shoot and root dry weight are presented in Table 2. The main effects and their interactions were highly significant ($P < 0.01$) for dry weight of roots and shoots. Results related to the influence of iron concentrations on dry weight of roots and shoots of 12 rice cultivars are presented in Table 3. Statistical differences were obtained among cultivars at all concentrations of Fe except for root dry weight at the highest level of Fe.

Dry matter of roots and shoots of all the cultivars decreased with increasing concentrations of Fe in the nutrient solutions, but varied from cultivar to cultivar. Averaged across cultivars, the decrease in the root weight was 52% and 68% at 890 and 1780 μM Fe compared with the optimum level. Similarly, the decrease in shoot weights was 71 and 74% at the two highest levels of Fe as compared to the optimum level. This means shoot weight was more sensitive than root weight to iron toxicity. Therefore, shoot weight was used as a criterion to classify rice cultivars for iron toxicity (Table 4). Shoot dry matter efficiency index was calculated to classify cultivar response to iron levels. This index is useful in separating high yielding, unstable and iron-susceptible cultivars. Cultivars with an efficiency index greater than 1 are considered to be Fe-tolerant, sensitive cultivars are in the range of 0–0.5 efficiency index, and cultivars in between these two limits are considered to have intermediate tolerance to iron toxicity. These ratings were selected arbitrarily.

Results in Table 4 show that 42% of the cultivars

Table 1. Activity of FeEDTA complex in the nutrient solution at different iron concentrations and pH (in μM)

Fe conc.	pH						
	4.0	4.5	5	5.5	6	6.5	7
90	79.5	89.6	79.6	79.6	79.6	79.7	79.8
890	786	787	788	788	788	788	789
1780	1570	1570	1570	1570	1570	1570	1580

Table 2. F-Values for analysis of variance of dry weight of roots and shoots

Source of variance	Dry wt roots	Dry wt shoots
Cultivars	2.97**	6.09**
Fe	200.79**	913.19**
CV × Fe	1.59**	3.78**

** Significant at the 0.01 probability level.

were tolerant at 890 μM Fe-level and 58% were moderately tolerant. Similarly, at the highest Fe-concentration, 33% of the cultivars were tolerant and 67% were moderately tolerant. The tolerant cultivars at the highest Fe levels were P3083F4-61, CNA1051, CNA3814, and P3304F4-27. Varietal variability in tolerance to iron toxicity may be related to differences in root oxidizing power of the rice cultivars. Rice roots can oxidize various compounds at the root surface and in the close vicinity of the roots (Ando *et al.*, 1983; Armstrong, 1969). The oxidizing power of rice roots functions as a defense mechanism against toxic substances such as ferrous iron and hydrogen sulfide in submerged soils. This means use of tolerant cultivars can be a complementary solution to reduce iron toxicity in flooded rice.

Simple correlation coefficients were determined among root and shoot growth and concentration tion of nutrients in the shoot (Table 5). Root and shoot weight were highly correlated. Root and shoot weights were positively correlated ($P < 0.01$) with concentrations of P, K, and Mn, but negatively correlated ($P < 0.01$) with concentrations of Ca, Cu, and Fe in the shoot. Among nutrients, some of the highest positive correlations ($P < 0.01$) were: N with P, P with K, Zn with P, K with Mg, Cu with Ca, and Mn with P. Iron con-

Table 4. Shoot dry matter efficiency index and classification of 12 rice cultivars for iron toxicity

Cultivars	Iron concentrations (μM)	
	890	1780
P3304F4-27	1.29 (T)	1.05 (T)
P3293F4-96-6	1.13 (T)	0.79 (MT)
CNA 3814	1.38 (T)	1.22 (T)
CNA 3882	0.71 (MT)	0.92 (MT)
P2915F4-2-1	0.67 (MT)	0.75 (MT)
P3083F4-61	1.48 (T)	1.59 (T)
CNA 0007	0.67 (MT)	0.69 (MT)
CNA 3084F4-59	0.89 (MT)	0.93 (MT)
CNA 1051	1.21 (T)	1.31 (T)
CNA 3739	0.87 (MT)	0.95 (MT)
CNA 3762	0.94 (MT)	0.95 (MT)
CNA 5193	0.85 (MT)	0.88 (MT)

$$\text{Dry Matter Efficiency Index} = \frac{\text{Yield at High Fe Level}}{\text{Exp. Mean Yield at High Fe Level}} \times \frac{\text{Yield at Optimum Fe Level}}{\text{Exp. Mean Yield at Optimum Fe Level}}$$

Optimum Fe level was 90 μM; T = Tolerant and MT = Moderately Tolerant.

Table 3. Dry weight of roots and shoots (in g per 4 plants) of 12 rice cultivars as influenced by iron concentrations in nutrient solution

Cultivars	Iron concentration (μM)					
	90		890		1780	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
P3304F4-27	0.65b	5.03a–c	0.46b	1.57ab	0.25a	1.13b
P3293F4-96-6	0.98ab	4.57b–d	0.58a	1.52ab	0.24a	0.94b
CNA 3814	1.11a	5.93a	0.43bc	1.42ab	0.23a	1.11b
CNA 3882	0.74ab	4.30b–d	0.30d–g	1.00b	0.26a	1.16b
P2915F4-2-1	0.75ab	3.64de	0.26fg	1.13b	0.25a	1.11b
P3083F4-61	0.77ab	4.89bc	0.41b–d	1.85a	0.26a	1.76a
CNA 0007	0.66b	3.14e	0.37b–f	1.31ab	0.26a	1.20b
CNA 3084F4-59	0.62b	4.77bc	0.25g	1.15b	0.24a	1.06b
CNA 1051	0.83ab	5.23ab	0.38b–d	1.41ab	0.27a	1.35ab
CNA 3739	0.75ab	4.76bc	0.27e–g	1.11b	0.23a	1.08b
CNA 3762	0.82ab	4.16cd	0.46b	1.38ab	0.22a	1.18b
CNA 5193	0.77ab	4.13cd	0.33c–g	1.25b	0.24a	1.16b

Means in the same column followed by the same letter are not significantly different at the $P = 0.05$ level by Duncan's Multiple Range Test.

Table 5. Simple correlation coefficients among root and shoot dry weight and nutrient concentrations in the shoots of 12 rice cultivars

Variables	Root wt	Shoot wt	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe
Root wt	1.00										
Shoot wt	0.89**	1.00									
N	0.13NS	0.21NS	1.00								
P	0.74**	0.77**	0.47**	1.00							
K	0.64**	0.67**	0.17NS	0.82**	1.00						
Ca	−0.51**	−0.51**	−0.19NS	−0.28NS	−0.16NS	1.00					
Mg	−0.02NS	0.01NS	−0.03NS	0.25*	0.32**	0.20NS	1.00				
Zn	0.20NS	0.24*	0.08NS	0.32**	0.40**	−0.04NS	0.34**	1.00			
Cu	−0.38**	−0.45**	0.26*	−0.12NS	−0.09NS	0.36**	0.19NS	−0.11NS	1.00		
Mn	0.31**	0.32**	0.03NS	0.53**	0.49**	0.17NS	0.34**	0.37**	−0.03NS	1.00	
Fe	−0.81**	−0.78**	0.08NS	−0.56**	−0.47**	0.46**	0.18NS	−0.06NS	0.48**	−0.33**	1.00

NS = Not significant; * and ** = Significant at 5 and 1% level or probability, respectively.

centrations were negatively correlated ($P < 0.01$) with P, K, Zn, and Mn. The negative correlation suggests a decrease in the uptake of these nutrients when Fe concentrations are high. Where iron toxicity is a problem in flooded rice, it may cause induced deficiencies of P, K, and Zn if levels of these nutrients are low. These results suggest that iron toxicity will be more severe under low fertility conditions as compared to high soil fertility.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 263–268.
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PLSO PN 163

Relationship between metolachlor sensitivity and Mn toxicity tolerance in sorghum cultivars

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Key words: acid soil, enzymes, gibberellin, glutathione, herbicide resistance, Mn-Mg ratio, *Sorghum bicolor* (L.) Moench

Abstract

Four sorghum [*Sorghum bicolor* (L.) Moench] cultivars were evaluated for metolachlor resistance and degradation. LD_{50} for TAM 428 and SC 599 exceeded 3 ppmw metolachlor. This was a $> 5\times$ increase in metolachlor resistance. SC 283 and GP 140 LD_{50}'s were 0.4 and 0.5 ppmw metolachlor which were $\sim 2\times$ as sensitive to metolachlor as the reference cultivar Funk G-522 DR. Degradation rates did not parallel resistance. TAM 428 (LD_{50} 3 ppm in bathing solution) had the same degradation rate as GP 140 (LD_{50} 0.5 ppm). Sorghum genotypic selectivity to metolachlor is not explicable as a differential herbicide degradation rate.

When grown in soil at pH 4.4, SC 599 was highly susceptible to excess Mn^{2+} (and possibly Al) and height and fresh weight were greatly decreased (86%). Aluminum toxicity was apparently not important according to soil Al values. TAM 428 was intermediate in response to excess Mn^{2+} (77% decreased height); while SC 283 and GP 140 were resistant to excess available Mn^{2+} and their growth was decreased 13 and 40%, respectively, due to acid soil stress. Mg/Mn ratios indicate that SC 599 absorbed Mn^{2+} or Mg^{2+} to the extent that they were available in the soil solution. The soil solution Mg/Mn ratio at low pH was the same as for the plants. But TAM 428, SC 283, and GP 140 appeared to have a mechanism to exclude excess Mn^{2+} that also decreased the absorption of Mg^{2+} so that Mg/Mn ratios remained uniform at low soil pH.

Differences in metolachlor and Mn^{2+} susceptibilities may be due to differential responses of the terpenoid biosynthesis enzyme systems which are strongly modified by both metolachlor and Mn^{2+}–Mg^{2+} concentrations.

Abbreviations. metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] cultivars vary in susceptibility to metolachlor (Wilkinson, 1988) but even 'resistant' cultivars may have stand reductions > 50% at normal metolachlor field concentrations (2.8 kg ha^{-1}) when unsafened (no herbicide antidote) seed are utilized. Sorghum tolerance to metolachlor has been presumed to result from more rapid met abolism of metolachlor via glutathione (GS) conjugation (Breaux, 1986; Breaux *et al.*, 1987; Fuerst, 1987; Jaworski, 1969; Zama and Hatzios, 1986). Yet, metolachlor inhibits gibberellin precursor biosynthesis (Wilkinson, 1981a) and exogenous GA induced an increased degradation of metolachlor into the GS-metolachlor conjugate (Wilkinson, 1988). Thus, selectivity among sorghum cultivars may involve physiological phenomenon other than metolachlor degradation.

In an extensive sorghum breeding program for suspected excess manganese tolerance (low level acid soil tolerance), cultivars were developed which were less susceptible to metolachlor (unpublished data). Therefore, evaluation of the mode-of-resistance for these cultivars was undertaken to ascertain whether stress tolerance *per se* was due to increased metolachlor degradation or other physiological processes.

Materials and methods

Bioassay

Twenty-five seeds of each sorghum cultivar (SC 599, TAM 428, Funk G-522 DR, GP 140, RTX 430, or SC 283) were placed into 10-cm petri dishes containing one circle (9 cm) Whatman No. 1 filter paper, 10 mL deionized water, and metolachlor (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, or 3.5 ppmw). After 96 h in the dark at 28°C, the roots were measured to the nearest mm. Ten roots/dish were measured and each treatment was replicated eight times. Average root lengths/dish were subjected to analysis of variance on a randomized complete block design. Mean separations were by the multiple range test at the 5% level.

^{14}C-*Metolachlor degradation*

Twenty-five sorghum (GP 140, SC 283, TAM 428, or SC 599) seeds were placed into petri dishes as described above without metolachlor. After 96 hr growth, 1-cm coleoptiles were collected, washed, blotted dry, weighed, and inserted into 3 mL solution containing 5 m*M* HEPES, 0.5 m*M* $CaCl_2$, 0.1 m*M* $MgCl_2$, 1.0 m*M* KCl, and 5 μM metolachlor (1.34 μCi μM^{-1}). After 2-hr incubation, the coleoptiles were separated from the bathing solution, washed with water, and extracted with absolute methanol. After filtration, the methanol was evaporated at 30°C on a rotary evaporator. The residue was liquified in 10 mL methanol. A 1-mL aliquot was taken for total ^{14}C content. Thin-layer chromatograms on silica gel g (0.25 mm) (activated at 100°C for 1 hr) with n-butanol:acetic acid:water (12:3:5, v, v, v) in a paper-lined tank were used to separate the components. One-cm strips of the silica gel were scraped into vials and ^{14}C content determined by liquid scintillation counting (Beckman LS-100) for 20 min or 1% accuracy.

Percentage of ^{14}C in each 1-cm strip was calculated using the total ^{14}C on each sample chromatogram as the base. Original ^{14}C-metolachlor was added to each TLC plate as a reference standard. Contents of ^{14}C-metolachlor (R_f 0.75) and (presumed) ^{14}C-metolachlor-GS conjugate (R_f 0.4–0.5) (6) were subjected to analysis of variance on a randomized complete block design. Mean separations were by the multiple range technique at the 5% level.

Soil pH evaluation

Cecil (Typic Hapludult) sandy loam was adjusted with lime ($CaCO_3$) to pH 4.4 or 5.6 corresponding to high and low Mn (low and high Al, respectively) availability. Twenty-five seeds of each cultivar were planted into the soil in peat pots (24 cm × 14 cm × 8 cm). Eight replications were used. The pots were subirrigated and after 21 days growth, the height and fresh weight of ten seedlings/cultivar/soil type/replication were measured for height (cm). Shoot tissue was weighed and analyzed for manganese and magnesium contents by atomic absorption. Data were analyzed on a randomized complete block design and means were separated by the multiple range test.

Specially constructed plastic centrifuge tubes holding about 250 mL, and having a separable reservoir at the bottom were packed with soil at near field moisture capacity; this soil had been kept moist at field capacity from the pH experiment. Soil solution was extracted by centrifuging for 2 hr at 2300 rpm. Soil pH was determined on a 1:2 soil to water suspension. Exchangeable soil Al was measured using N KCl (Barnhisel and Bertsch, 1982). Soil samples (4.0 g) were shaken with 20 mL water for 30 min. and the supernatant separated by centrifuging and filtering to measure water-extractable Mn. Cation exchange capacity (CEC) was measured by summing the bases extractable with *M* NH_4OAc (pH 7.0) and the KCl-extractable Al. Percent Al saturation was calculated by dividing KCl Al by the CEC and multiplying by 100. The Aluminon procedure (Hsu 1963) was used to

analyze Al in the KCl extracts, and atomic absorption or emission spectroscopy was used for the other elements. The ionic strength of the soil solution was estimated by measuring the electrical conductivity and multiplying by a constant (Griffin and Jurinak, 1973). The extended Debye-Hückel equation was then employed to calculate the chemical activity of Mn and Mg in the soil solutions.

Results and discussion

Bioassay

Two sorghum cultivars (SC 599 and TAM 428) were resistant to metolachor (Fig. 1) with 80% growth (root elongation) of the untreated check at 3 ppmw. When compared to a cultivar with traditional metolachlor susceptibility (Funk G-522 DR) (Fig. 1) (Wilkinson, 1981b), this approximates a $> 5\times$ increase in resistance to metolachlor. Two cultivars (GP 140 and SC 283) were highly susceptible to metolachlor (Fig. 1) and were approximately twice as susceptible as Funk G-522 DR (Fig. 1). Thus, metolachlor resistant and susceptible cultivars were available for ^{14}C-metolachlor metabolism studies.

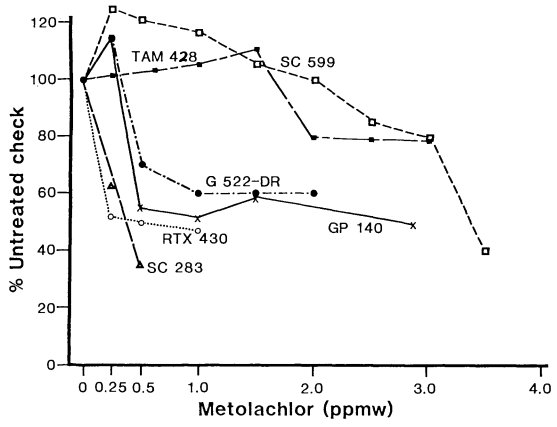

Fig. 1. Metolachlor influence on cultivar root elongation.

^{14}C-*Metolachlor degradation*

Total ^{14}C concentrations in coleoptiles (DPM g FW^{-1}) was greatest in the SC 599 cultivar (Table 1) while the other three cultivars contained approximately the same total ^{14}C concentration. On the basis of total ^{14}C-metolachlor concentration in the coleoptiles (DPM g FW^{-1}), TAM 428 and SC 599 were equivalent (Table 1). But GS-^{14}C-metolachlor was greatest in SC 599; while TAM 428 and GP 140 were equivalent.

On a percentage basis, although SC 599 absorbed more total ^{14}C (DPM g FW^{-1}), the residue as parent ^{14}C-metolachlor was significantly lower than in the other three cultivars (Table 1). TAM 428 and GP 140 contained equivalent total ^{14}C, % ^{14}C-metolachlor, % GS-^{14}C-metolachlor and 'other' ^{14}C-metolachlor degradation products (Table 1). SC 283 contained significantly higher concentrations of ^{14}C-metolachlor and lower concentrations of associated degradation products.

Differences in ^{14}C-metolachlor degradation patterns can be seen in Table 2. SC 599 contained

Table 1. Parent ^{14}C-metolachlor and degradation products extracted from 96-hr sorghum coleoptiles (etiolated) after 120 min exposure

Cultivar	^{14}C-metolachlor	GS-^{14}C-metolachlor	Other products	Total
DPM g^{-1} fresh weight				
TAM 428	1209.6a[a]	483.1b	413.9b	2367.5
SC 599	1240.2a	1166.4a	852.4a	3464.3
SC 283	1766.0a	285.5c	290.6b	2350.2
GP 140	1621.5a	567.5b	485.3b	2680.2
% DPM on TLC plate				
TAM 428	59.4b	20.6b	17.5a	
SC 599	35.3c	33.8a	24.0a	
SC 283	73.9a	11.9c	13.7a	
GP 140	60.4b	21.4b	18.0a	

[a] Values in a column in a box followed by the same letter are not significantly different at the 5% level according to the multiple range test.

Table 2. Percentage of total DPM on thin-layer chromatography of 96-hr old etiolated sorghum coleoptiles treated with ^{14}C-metolachlor for 2-hr. Each value is the average of five replications

cm	R_f	TAM 428	SC 599	SC 283	GP 140
15	1.0	2.0a[a]	0.1a	0.2a	0.2a
14		0.2a	0.2a	0.2a	0.3a
	0.9				
13		0.2a	9.0a	6.9a	8.6a
12	0.8	10.2b	16.7a	43.8a	45.5a
11		40.9a	3.9c	21.4b	6.2c
	0.7				
10		7.5a	3.6b	4.7b	3.8b
9	0.6	5.5a	4.7a	3.6a	5.1a
8		4.8b	16.8a	7.5b	7.9b
	0.5				
7		10.1a	7.1b	2.5c	6.2b
6	0.4	4.5bc	9.9a	2.3c	5.4b
5		4.1ab	6.6a	1.5b	4.2ab
	0.3				
4		2.4ab	2.2ab	1.1b	2.5a
3	0.2	1.5b	2.7a	1.2b	1.2b
2		1.5b	4.7a	1.2b	1.4b
	0.1				
1		2.2b	4.2a	1.0c	0.7c

[a] values in a line followed by the same letter are not significantly different at the 5% level according to the multiple range test.

significantly different degradation products at R_f 0.4 and 0.1 (Table 2). These products may have been present in TAM 428 and GP 140 but appeared to be absent in SC 283.

Comparison of metolachlor resistance (susceptibility) (approximate LD_{50}) and percent total ^{14}C-metolachlor degradation showed very little correlation between susceptibility and degradation (Table 3). SC 599 had the greatest metolachlor degradation and a high metolachlor resistance (Table 3) and this was particularly present when SC 599 was compared to SC 283. But TAM 428 had metolachlor resistance equivalent to SC 599 (Fig. 1) (Table 3) and did not degrade metolachlor as rapidly as did the cultivar SC 599 (Tables 2 and 3). Additionally, TAM 428 and GP 140 were equivalent in ^{14}C-metolachlor uptake and degradation but there was a massive difference in metolachlor susceptibility (Fig. 1, Tables 1, 2 and 3).

Table 3. Comparison of cultivar susceptibility to (root growth) and degradation of (etiolated coleoptile) metolachlor by four sorghum genotypes

Cultivar	Degradation (%)	LD_{50} (ppmw)
TAM 428	38.1	> 3.0
SC 599	57.8	> 3.0
SC 283	25.6	~ 0.4
GP 140	39.4	~ 0.5

Thus, although selectivity among different plant species might be determined by the ability to degrade the herbicide, selectivity within a species was not solely a factor of herbicide degradation. Some other physiological factor must be determinative.

Soil pH evaluation

Cultivar heights in the low pH soil were SC 283 = GP 140 > TAM 428 > SC 599 (169, 168, 109, and 46 cm, respectively) (Table 4). Growth of the SC 599 in the low pH soil was severely stunted and had massive Mn concentrations as well as a high Mg concentration. Therefore, the data indicate that SC 599 absorbed whatever ions were available in the soil solution, but that the other three cultivars were able to exclude excess Mn from being absorbed. When the plants were grown in a high pH soil, the manganese was of limited availability (Table 5) and the magnesium concentration of the plants was roughly equivalent. These concepts are best illustrated by the Mg/Mn ratios.

Mg concentration in SC 599 was roughly equivalent at both soil pH's, but Mn concentration was greatly increased in plants grown in a low pH. These concentrations of Mn are generally considered to be toxic to sorghum (Galvez *et al.*, 1987). Thus, the Mg/Mn ratios for low and high soil pH grown plants was 0.5 and 51.9, respectively. Mg/Mn ratios in all four cultivars were 0.5 for plants grown in the low pH soil. When the plants were grown in a high pH soil, the Mg/Mn ratios varied from 33.0 to 51.9. This is a direct measurement of Mg and Mn availability to the plant when the plants were grown in a high pH soil.

At low soil pH values, not only does Mn become high in availability, but Al and other metals also increase. The possibility of Al toxicity interactions in the soil pH experiment must be considered. In field trials over many years it has been observed that this soil must be below pH 4.4 to cause severe Al toxicity reactions in sorghum (Duncan, 1988).

Table 4. Relationship between Mg contents and growth of sorghum cultivars grown in soil at two different soil pH's

	pH 4.4			pH 5.6			Mg/Mn	
	Ht (mm)	Mn (mg g DW^{-1})	Mg	Ht (mm)	Mn (mg g DW^{-1})	Mg	Low	High
SC 599	46c	7.73a	1.72a	293b	0.088a	2.02b	0.5a	51.9a
SC 283	169a	3.32bc	0.84bc	195c	0.121a	1.97b	0.5a	37.0bc
GP 140	168a	2.89c	0.67c	283b	0.170a	2.49a	0.5a	33.0c
TAM 428	109b	4.06b	1.06b	464a	0.143a	2.76a	0.6a	43.6b

[a] Values in a column followed by the same letter are not significantly different at the 5% level according to the multiple range test.
[b] Ratios were calculated on μmol g DW^{-1} basis.

Table 5. Soil pH, exchangeable Al, percent Al saturation, extractable Mn, and soil solution Mn and Mg chemical activity for a Cecil soil amended with two lime levels

Lime trt	Soil pH (H_2O)	KCl exch. Al ($cmol_c\,kg^{-1}$)	Al saturation (% of CEC)	H_2O-extr. Mn ($\mu g\,g\,soil^{-1}$)
Low	4.41b[a]	0.95a	48.6a	1.70a
High	5.64a	0.04b	1.6b	0.18b
	Chemical activity in soil solution			
	Mn (μmol L^{-1})	Mg (μmol L^{-1})	Mg/Mn ratio	
Low	63.9a	46.9a	0.7b	
High	1.8b	46.9a	26.1a	

[a] Values followed by the same letter within a column and variable are not different according to F ratios from Analysis of Variance.

The low pH here was 4.41 (Table 5). Exchangeable Al values (Table 5) were not high enough to cause appreciable Al toxicity (Adams and Lund, 1966). The Al saturation was also below the usual critical level of 50% for this type of soil (Table 5) and for sorghum (Borgonovi *et al.*, 1987). These values along with the fact that Mn toxicity symptoms were evident in the plants suggests that only Mn toxicity was important.

Soil water-extractable Mn decreased nearly one log unit between the low pH and high pH soils, and the soil solution Mn decreased 1.55 log units (Table 5). We would expect a theoretical decrease of two log units in Mn chemical activity for a one unit increase in pH (Lindsay, 1979). Since the pots were subirrigated, at least portions of them were probably not well aerated. Thus, the redox parameter pe + pH was most likely lower than 16.62 (pyrolusite-manganite equilibrium) (Lindsay, 1979). This redox could be conducive to Mn solubilization especially on low pH soil. The soil solution Mg was not different between treatments leading to large differences in the Mg/Mn ratios (Table 5). The ratio for low pH soil was comparable to the ratio in the plants at low pH (Table 4) showing that the plants did respond to the chemical activity of Mn and Mg in the soil solution. The soil ratio at high pH was somewhat lower than for the plants.

Height is an integrated response to toxic concentrations of Mn and possible Mg deficiency. The isoprenoid biosynthesis system has at least three enzymes which use Mn or Mn $\pm$ Mg. Products of the isoprenoid biosynthetic system include chlorophyll, carotene, plastidic quinones, and gibberellic acids (GA). These Mn-utilizing enzymes are highly susceptible to excess Mn, and their activities are decreased 2–10 $\times$ at Mn $> 5\,\mu M$. Loss of plastidic pigments and electron carriers decreases photosynthesis. Decreased synthesis of GA is equivalent to stunting. Thus, the influence of the high Mn content of SC 599 grown at a low pH is explicable as a decrease in isoprenoid biosynthesis activity.

Whether that decrease in isoprenoid biosynthesis is correlated with total GA biosynthesis or the other two enzymes is unkown. Ultimately comparative biosynthetic assays of the activities of

these enzymes in relation to metolachlor and Mn ± Mg concentrations is warranted. The influence of Al toxicity on these enzyme systems should also be investigated.

Acknowledgments

This work was conducted from funds allocated to Projects 1306, 1408, and 1410 State and Hatch of the University of Georgia; College of Agriculture Experiment Station. Thanks are gratefully extended to Dr H LeBaron, Ciba-Geigy for ^{14}C-metolachlor.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 269–271.
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PLSO PN 164

Genetic studies on the acidification capacity of sunflower roots induced under iron stress

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Key words: *Helianthus annuus*, inheritance, iron stress, proton efflux, sunflower

Abstract

Under stress of iron deficiency roots of sunflower (*Helianthus annuus* L.) increase proton efflux which acidifies the root medium, increase the ferric reducing capacity and the exudation of phenolic compounds. Differences have been found previously among sunflower inbred lines in the capacity of their roots to lower pH and it was also found that this character is under genetic control.

This work presents the results of an inheritance study made by crossing two genotypes, one (CMS HA 89) without acidification capacity and another (RHA 271) with it. Plants were grown individually in 75 mL vessels with an aerated solution low in iron. After 4 days, solutions were changed to one without iron and the pH of the medium was measured during the following days. Results from F_1, F_2, and backcross generations can be explained with two pairs of alleles controlling the character, being the relation between alleles of complete dominance at both gene pairs, but either gene, when dominant is epistatic to the other.

Introduction

Sunflowers, as many other dicotyledonous species, develop under iron deficiency cytological, morphological and physiological changes in their roots which increase iron uptake and translocation. These responses in sunflower involve differentiation of peripheral cells into transfer cells and development of abundant root hairs in a swollen zone near the root apex, increase in the ferric reducing capacity, proton efflux and release of compounds with capacity to chelate and reduce ferric ions (Kramer *et al.*, 1980; Römheld and Marschner, 1979). Römheld and Marschner (1986) discuss the importance of these responses in dicotyledonous species and compare them with the responses of graminae.

Genetic studies on iron efficiency have been made in several species: soybean (Cianzio and Fehr, 1982; Weiss, 1943), tomato (Wann and Hills, 1973) and bean (Coyne *et al.*, 1982).

In previous work we have found differences among sunflower inbred lines in the acidification capacity and that this character was under genetic control (Alcántara and De la Guardia, 1986; 1987). The purpose of this work was to study the inheritance of this character in several generations derived from the cross between two inbred lines of sunflower with different acidification capacity under iron deficiency.

Materials and methods

The original parental plant materials used in this study were the American lines CMS HA 89 (P_1) and RHA 271 (P_2). Line CMS HA 89 is cytoplasmic male sterile and line RHA 271 is a fertility restorer, both lines are used to produce commercial hybrids. Under iron deficiency stress, line CMS HA 89 showed a uniform response without acidification capacity of the roots medium. Line RHA 271, although it had acidification capacity, had an uneven response, it was then selected for several

generations until a uniform acidification capacity was obtained. Afterwards, F_1, F_2, $BC_1(P_1 \times F_1)$ and $BC_2(P_2 \times F_1)$ generations were obtained in a greenhouse by hand pollination and only emasculation of line RHA 271 was needed to get the BC_2 generation. To test the acidification capacity, seeds treated with sodium hypochlorite (0.5% v/v) were germinated in the dark for 2 days in perlite moistened with 5 m*M* $CaCl_2$, seeds with well-developed radicles were transferred to plastic netting held over one-half strength standard nutrient solution for 2 more days in the dark. Seedlings were then transplanted individually to plastic vessels containing 75 mL of continuously aerated standard solution with the following composition: 2 m*M* $Ca(NO_3)_2$, 0.75 m*M* K_2SO_4, 0.25 m*M* KH_2PO_4, 0.65 m*M* $MgSO_4$, 50 μM KCl, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.05 μM $(NH_4)_6Mo_7O_{24}$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$ and 5 μM Fe EDDHA. The pH was adjusted to 6.0 with KOH 0.1 *N*. Plants were grown in a growth chamber at 22°C day/18°C night, with a 14 h photoperiod, relative humidity between 60 and 80% and a photosynthetic irradiance of 350 $\mu mol\, m^{-2} s^{-1}$ provided by fluorescent tubes (Sylvania cool white VHO). After 4 days, the solution was changed to a new standard solution without Fe and pH 6.0 and the roots were rinsed with distilled water. The pH of the root medium of each plant was measured every day from the second day without Fe, until a pH $\leqslant$ 4 was attained. The medium was renewed at day 5 and 10 without Fe.

The agar bromocresol purple technique used by Marschner *et al.* (1982) to localize the acidification capacity along the root was also used with some plants. In the chi square analysis the Yates correction was applied (Strickberger, 1968).

Results

The nutrient solution used had NO_3 as N source, and absorption of NO_3-N usually causes the pH of the solution to rise. However, the H ion excretion under Fe stress was such that the bulk nutrient solution pH decreased to 4 or under in a few days in plants with acidification capacity. Roots of plants which did not lower pH became necrotic and these were classified as without acidification capacity and discarded. Plants that did not lower pH after 12 days without Fe also were classified as without acidification capacity.

In the agar test, the plants with capacity to acidify the nutrient solution showed a high capacity located in the subapical root zones, as visualized by the strong yellow color produced. Some of the plants without capacity to acidify the nutrient solution showed a very light yellowish colour in the same locations. Plants classified as without acidification capacity may have a small power to acidify, but the difference established between the two classes is wide enough.

The distribution of plants for acidification capacity in the different generations is shown in Table 1. All plants of the F_1 and BC_2 generations had acidification capacity, while in the F_2 and BC_1 generations there was segregation with frequencies of plants without acidification near $\frac{1}{16}$ and $\frac{1}{4}$ respectively. Thus the acidification capacity appears to be dominant with 2 genes involved, so that only plants with the 2 pairs of recessive alleles have no acidification capacity. Results of chi square tests (Table 2) show that this hypothesis is reasonable.

Discussion

The mechanisms of resistance to iron deficiency chlorosis are complex and not yet fully understood. It is not surprising that different genetic control appeared to be dependent on the cultivars, species and test conditions used to evaluate this character. Weiss (1943) in soybean and Wann and Hills (1973) in tomato found that susceptibility to iron chlorosis was determined by a single recessive gene. Later studies in soybean (Cianzio and Fehr, 1982) showed the existence of a more complex system,

Table 1. Number of plants distributions for acidification capacity in several generations

Generation	Acidification capacity		
	With	Without	Total
P_1 (CMS HA 89)	0	34	34
P_2 (RHA 271)	37	0	37
F_1 (CMS HA 89 × RHA 271)	62	0	62
BC_1 (CMS HA 89 × F_1)	134	42	176
BC_2 (RHA 271 × F_1)	30	0	30
F_2 (F_1 × F_1)	88	6	94

Table 2. Chi square tests for acidification capacity in BC_1 and F_1 generations

Generation	Acidification capacity				x^2	P
	Observed		Expected			
	With	Without	With	Without		
F_2	88	6	88.125	5.875	0.026	0.90–0.75
BC_1	134	42	132	44	0.068	0.90–0.75

with quantitative inheritance and additive effects. In bean, Coyne *et al.*, (1982) also found quantitative inheritance, with complete dominance for resistance in two crosses, and they suggested that two major gene pairs were involved. In the present work only one of the mechanisms of the iron stress response has been studied genetically: the acidification capacity. The inheritance of the character can be explained with two pairs of alleles controlling the character, being the relation between alleles of complete dominance at both gene pairs, but either gene, when dominant is epistatic to the other (Strickberger, 1968).

Differences exist among species considered iron-efficient in the acidification capacity (Chaney, 1987; Römheld and Kramer 1983). This response has been related to the capacity to form transfer cells (Kramer *et al.*, 1980; Landsberg, 1986; Römheld and Kramer, 1983), and may contribute to iron efficiency through: (a) mobilization of low soluble ferric compounds, (b) increase in the reductase activity and (c) increase in the release of compounds which chelate ferric ions facilitating its diffusion to the root cells. (Barret-Lennard *et al.*, 1983; Ric de Vos *et al.*, 1986; Römheld and Marschner, 1979; 1986).

In the present work we have characterized the genetic control of the acidification capacity in two genotypes of sunflower, however the genetic control of other Fe stress responses, such as the increase in reducing capacity is unknown. The knowledge of all mechanisms involved in the Fe stress response and their genetic control will be very useful to select better genotypes for iron efficiency.

Acknowledgements

The work was supported by Comision Interministerial de Ciencia y Tecnología, Project PA 86-337.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 273–280.
PLSO PN 165
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Screening for manganese efficiency in barley (*Hordeum vulgare* L.)

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Key words: barley, chlorosis, deficiency, *Hordeum vulgare* L., manganese, screening

Abstract

Because Mn deficiency is a chronic problem in clacareous soils in South Australia, Mn efficiency (the ability to maintain yield on soils with limited Mn availability) has become one objective in the barley (*Hordeum vulgare* L.) breeding program at the Waite Institute. A screening method has been developed for use in the growth cabinet. Soil collected from a site with a history of Mn deficiency and stored air-dry is incubated at field capacity in a growth cabinet for 3 weeks prior to sowing. Plants are then grown in the growth cabinet at 15/10 °C, with 10 hour days. When the barley is tillering, plants are visually scored for Mn-deficiency symptoms.

The genotypes which are the most Mn-efficient in the field (based on grain yield) show the least development of Mn-deficiency symptoms in the growth cabinet. Visual scores separate the efficient and inefficient genotypes better than Mn concentration or content or leaf fluorescence.

Once severe Mn deficiency has developed on the least efficient genotypes, all of the plants are cut back to ground level and allowed to regrow in the growth cabinet. The least efficient genotypes die while efficient genotypes produce relatively vigorous regrowth and can be repotted into Mn-adequate soil and grown to maturity. A combination of the visual score before cut-back and assessment of the regrowth is used to distinguish the genotypes which show the most promise. Preliminary results using this method to examine the inheritance of Mn efficiency indicate that it is controlled by a single, major, dominant gene.

Introduction

Manganese (Mn) deficiency is a problem on calcareous soils in South Australia and can result in serious yield loss. The agronomic method of controlling Mn deficiency is to apply $MnSO_4$ at sowing and to supplement that with foliar applications of Mn at tillering. Fertilizer Mn application is not completely satisfactory since there is little residual value of applied Mn and the foliar sprays increase the number of farmer operations. Also, there are frequently periods before spray applications in which the crop is Mn deficient. As there is evidence that nutrient deficient plants are more susceptible to disease (Graham, 1983), yield losses would be best minimized by completely avoiding sub-clinical and marginal deficiencies.

Another method of minimizing yield losses caused by Mn deficiency is to grow a crop which is more Mn-efficient (is able to maintain yield on soils with limited Mn availability; for discussion, see Graham, 1988). Because barley is less susceptible to Mn deficiency than wheat (*Triticum aestivum*, L.) (Graham *et al.*, 1983; Nyborg, 1970), it is frequently the crop of choice in areas prone to Mn deficiency. Within barley, genotypic variation exists for Mn efficiency (Graham *et al.*, 1983; Sparrow *et al.*, 1983). Some current cultivars such as Weeah are relatively Mn-efficient. However, Weeah is not as high yielding as more recently released cultivars and lacks other desirable traits such as resistance to cereal cyst nematode (CCN). Increasing the Mn efficiency of higher yielding, CCN-resistant lines has become one objective in the barley breeding program at the Waite Institute.

Preliminary screening of barley lines for Mn

efficiency has been done in the field (Graham *et al.*, 1983). The strong influence of environmental conditions on severity of Mn deficiency (Reuter *et al.*, 1988) as well as the limitations of screening one generation per year cause problems with screening in the field. The controlled environment in a growth cabinet minimizes variation in severity of Mn deficiency due to environmental factors. It also provides the opportunity to intensify Mn deficiency by growing the plants in cool temperatures. Thus, these studies have concentrated on developing a screening method for use in the growth cabinet by the barley breeders in evaluating barley genotypes for increased levels of Mn efficiency. Visual scores of Mn-deficiency symptoms, leaf Mn contents and concentrations and leaf fluorescence are assessed as potential measurements of Mn efficiency in barley. Results from growth cabinet experiments are compared with ranking of barley lines based on grain yield in field trials. An example of the use of the method is also given in a screening experiment which examined the F_2 of a cross between an efficient and inefficient genotype.

Materials and methods

For the ranking experiments, 10 barley cultivars and breeder's lines were selected, based on known differences in Mn efficiency from earlier field work. Of these, WA 73s276 (WA), Weeah (WEE) and Stirling (STR) are the most Mn-efficient. O'Connor (O'C), Clipper (CLP) VIC 77015 (VIC) and Forrest (FOR) are intermediate. Schooner (SCH), Galleon (GAL) and WI 2584 (WI) are the least Mn-efficient. The range of Mn concentration in the seed for the growth cabinet experiment was 10.7 to 13.0 mg kg^{-1} dry weight. Seed was taken from the same source because it has been shown that seed Mn can affect the ranking of genotypes (Uren *et al.*, 1988) and barley growth and development (Longnecker *et al.*, 1988).

For the screening experiment, WEE and the Mn-inefficient WI 2585 were crossed. The parents and F_2 were grown and screened for Mn efficiency in the growth cabinet as described below.

The field trial was conducted at Wangary, SA, at a site with a history of Mn deficiency. Soil (80–90 % $CaCO_3$, pH = 8.4) was collected from this site for growth cabinet experiments. It was air-dired, sieved and stored. Prior to sowing the growth cabinet experiments, soil was incubated in the growth cabinet for 2—3 weeks at 25 % water content (w/w, approximately field capacity). This incubation is described in Uren *et al.* (1988). It increases the severity of Mn deficiency, possibly by stimulating microbial oxidation of Mn.

Field experiment

The experiment was designed as a complete randomized block, with four replicates. Plots were 20 m by 1.5 m and were split into two 10 m plots for receiving + and − Mn treatments. Seed was sown at Wangary, SA on 26 May, 1986, at 55 kg ha^{-1} with the − Mn plots receiving 298 kg ha^{-1} superphosphate with Cu, Zn, Mo and Co. The + Mn treatment received 250 kg ha^{-1} superphosphate with Mn (40 mg ha^{-1}), Cu, Zn, Mo, and Co, as well as two foliar applications of Mangasol™ at the recommended rates of 6.5 L ha^{-1} at early and mid-tillering. Plots were harvested 23 November.

Growth cabinet experiments

Barley seeds were pre-germinated on moistened filter paper in petri dishes and were then sown in pots containing 160 g of incubated soil to which the following nutrients had been added. (g kg^{-1} soil): 0.432 $Ca(NO_3)_2$ $4H_2O$; 0.190 KNO_3; 0.136 KH_2PO_4; 0.152 $MgSO_4$ $7H_2O$; (mg kg^{-1}): 11.6 $FeSO_4$ $7H_2O$; 1.04 HBO_3; 8.33 $CuSO_4$ $5H_2O$; 10.4 $ZnSO_4$ $7H_2O$; 1.04 $CoSO_4$ $7H_2O$; 7.8 NaCl; 0.104 H_2MoO_4 $4H_2O$; 1.04 $NiSO_4$ $7H_2O$. Mn treatments were nil added Mn (− Mn) and 100 mg Mn kg^{-1} soil, added as $MnSO_4$ (+ Mn).

Five replicate pots with two plants per pot were used for the ranking experiment. For the screening experiment, four pots of each parent, WEE and WI 2585, and 80 pots of the F_2 were used with one plant per pot. Black polyethylene beads were added to the soil surface to minimize evaporative water loss. Pots were watered to weight once or twice a week; for intermediate watering, identical amounts of water, determined by average water loss, were added to each pot. Plants were grown at 15/10 °C, with 10 hour days, midwinter conditions in South Australia. The light source was a mixture of high pressure metal halide and incandescent bulbs and

provided 500 μeinsteins $sec^{-1} m^{-2}$ at pot level. At early tillering, 2.5 mg N as KNO_3 and 2.5 mg N as $Ca(NO_3)_2$ were added per pot.

As soon as symptoms of Mn deficiency were observed, plants were visually scored on a scale of 1 to 5, with 1 = healthy, dark green plants, 2 = chlorotic plants, 3 = chlorosis and some necrotic spotting and/or interveinal chlorosis, 4 = severe chlorosis and necrosis and 5 = dead growing points. Scoring was repeated as symptoms continued to develop. Fluorescence was measured within one day of scoring as in Kriedemann *et al.* (1985) at the base of the youngest expanded blade with ligule showing (YEB) and the next oldest leaf (YEB + 1). After six weeks of growth, plants in the ranking experiment were cut back to ground level. The YEB and YEB + 1 were sampled for analysis of Mn content and concentration. After two weeks of regrowth, the plants were harvested and dry weights of roots and shoots measured. After six weeks in the screening experiment, the YEB and YEB + 1 were sampled, but the plants were not cut back since seed was desired for genetics studies. The YEB and YEB + 1 were dried for at least 24 hours in a forced air oven at 70 °C. They were digested in concentrated HNO_3 and analyzed using an inductively-coupled plasma emission spectrometer.

Results

The ranking of the 10 barley lines based on grain yield at a Mn-deficient site is given in Figure 1. In order to minimize the effect of factors other than Mn-deficiency stress on yield, this ranking is based on relative grain yield (i.e. [yield in − Mn plots/ yield in + Mn plots] × 100). The ranking is the same if based on yield in − Mn plots with the exception that VIC and CLP are reversed (data not shown).

Figure 2 shows the ranking of the genotypes in the growth cabinet based on visual score (Fig. 2a), Mn concentration in the YEB (Fig. 2b) and leaf fluorescence (Fig. 2c). The order of the genotypes on the x axis is based on the ranking in the field (see Fig. 1). The − Mn treatment causes severe Mn-deficiency symptoms, a decrease in YEB Mn concentration and an increase in leaf fluorescence of all genotypes examined (Fig. 2).

The two most efficient lines in the field were those which exhibited the least severe Mn-deficiency symptoms in the growth cabinet (Fig. 2a). By contrast, while the Mn-efficient lines generally have higher Mn content than the Mn-inefficient lines, the lines cannot consistently be separated using this measurement (note FOR in Fig. 2b). The results using Mn concentration ($\mu g\, g^{-1}$ dry weight) in the YEB and YEB + 1 and using Mn content (ng Mn $leaf^{-1}$) in YEB + 1 show the same trend (data not shown). Fluorescence of the YEB of all lines receiving no added Mn is much higher than the fluorescence of those lines receiving adequate Mn (Fig. 2c), but differences between lines were inconsistent on the different days when fluorescence was measured and this measurement did not separate Mn-efficient and inefficient lines. The fluorescence of YEB + 1 was not better for separating genotypes (data not shown). After cutting the plants back to ground level, the Mn-efficient lines produced the most vigorous regrowth (Fig. 3).

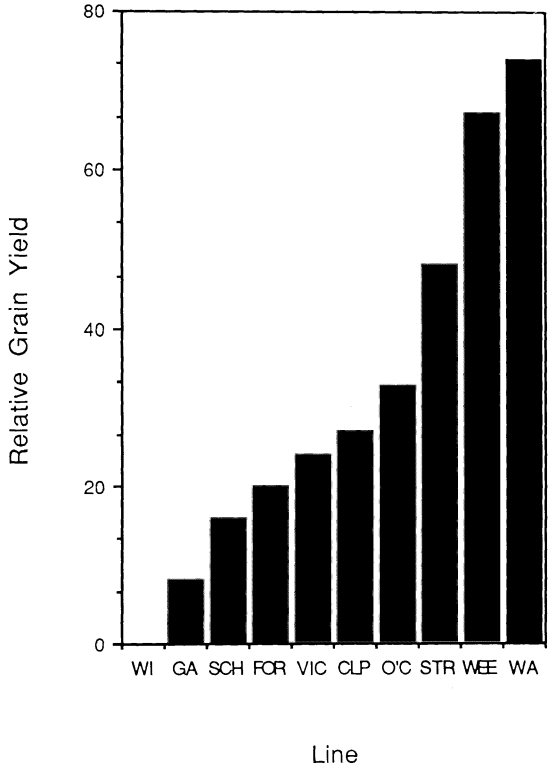

Fig. 1. Ranking of ten barley lines for Mn efficiency, based on relative grain yield in the field. ([yield in − Mn plots/yield in + Mn plots] × 100).

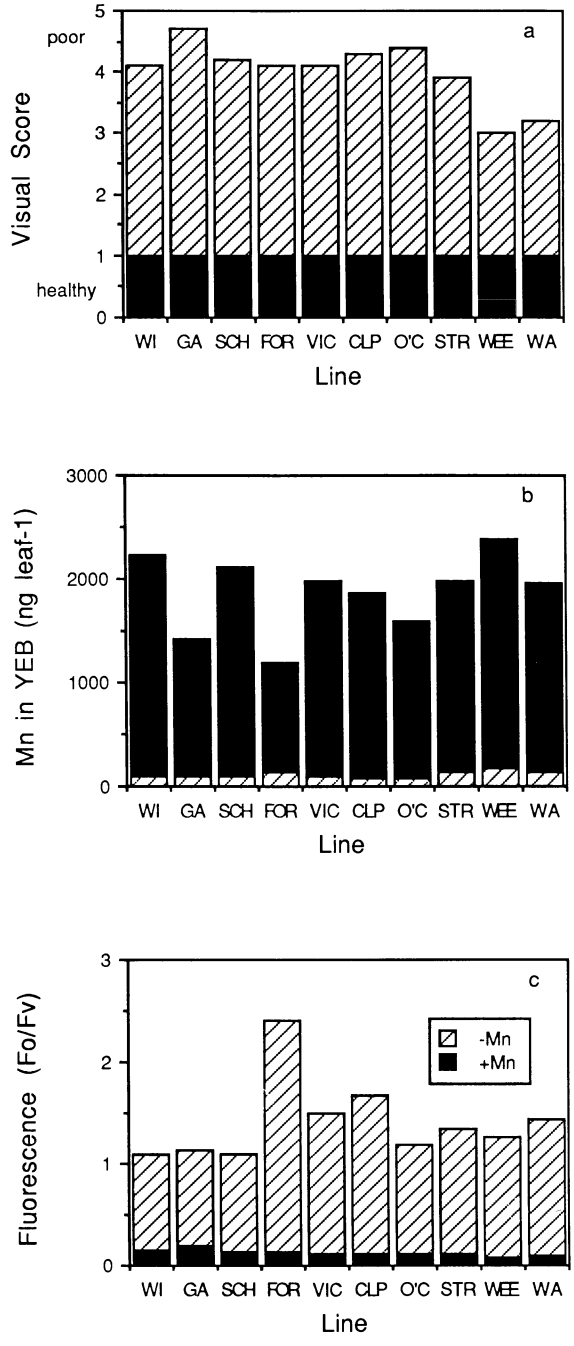

Fig. 2. Ranking of ten barley lines based on visual score (**a**), Mn content of YEB (**b**) and leaf fluorescence (**c**) of plants grown in the growth cabinet for six weeks. The order of genotypes on the x-axis is the same as in Figure 1.

The relationship between Mn deficiency symptoms (visual scores) and leaf Mn content is plotted in Figure 4a and between visual scores and leaf fluorescence in Figure 4b. For both, there is a good correlation with $r^2 = 0.87$ and 0.89, respectively.

Figure 5 shows the results of screening the F_2 generation of the cross between the Mn-efficient WEE and inefficient WI 2585 using visual scores (Fig. 5a) and Mn content (Fig. 5b) after six weeks growth. There was a greater relative separation between the visual scores of the 2 parents than between their YEB Mn content. WI 2585 showed severe deficiency symptoms with death of some growing points (scores 4.5—5) while WEE exhibited chlorosis, but only slight signs of necrosis (scores 2—2.5). The Mn contents of YEBs of WI 2585 were generally lower than of WEE, but did overlap somewhat. In this experiment, there was also a correlation between visual score and Mn content with $r^2 = 0.64$ (Fig. 6).

Discussion

For a screening technique to be successful, the

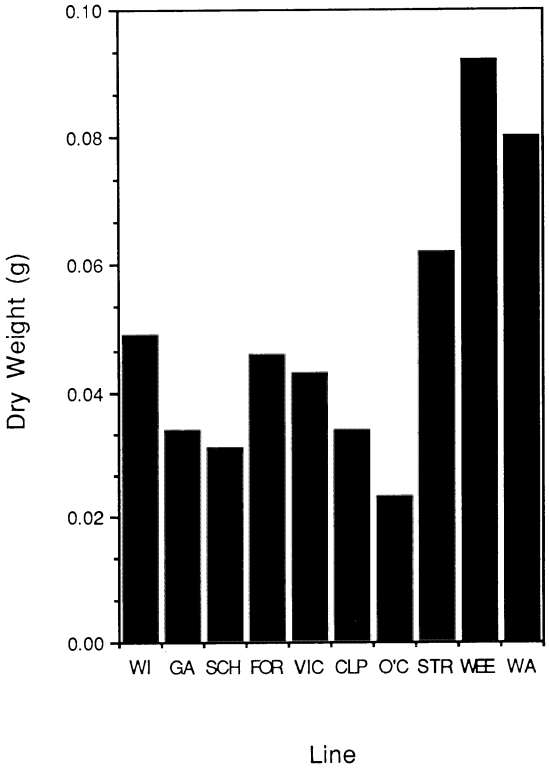

Fig. 3. Ranking of ten barley lines based on regrowth of plants after being cut back to ground level. Plants were grown in Mn-deficient soil in the growth cabinet for six weeks, cut back to ground level and then allowed to regrow for two weeks.

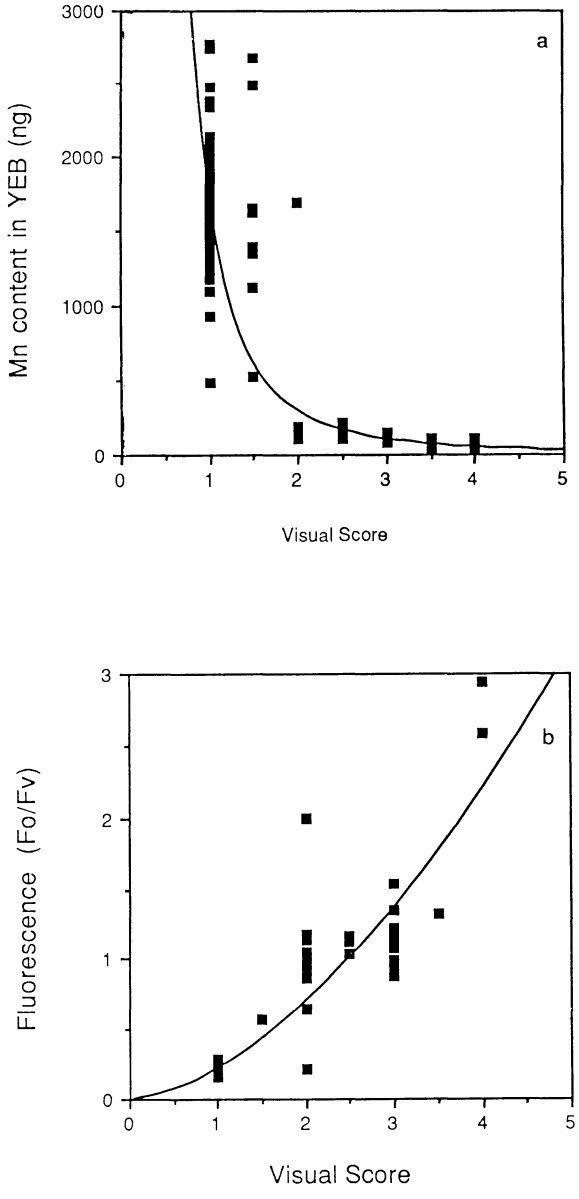

Fig. 4. Relationship between Mn-deficiency symptoms (visual scores) and leaf Mn content (ng Mn $leaf^{-1}$) (**a**) and between visual scores and leaf fluorescence (**b**). R^2 for a = 0.87, for b = 0.89.

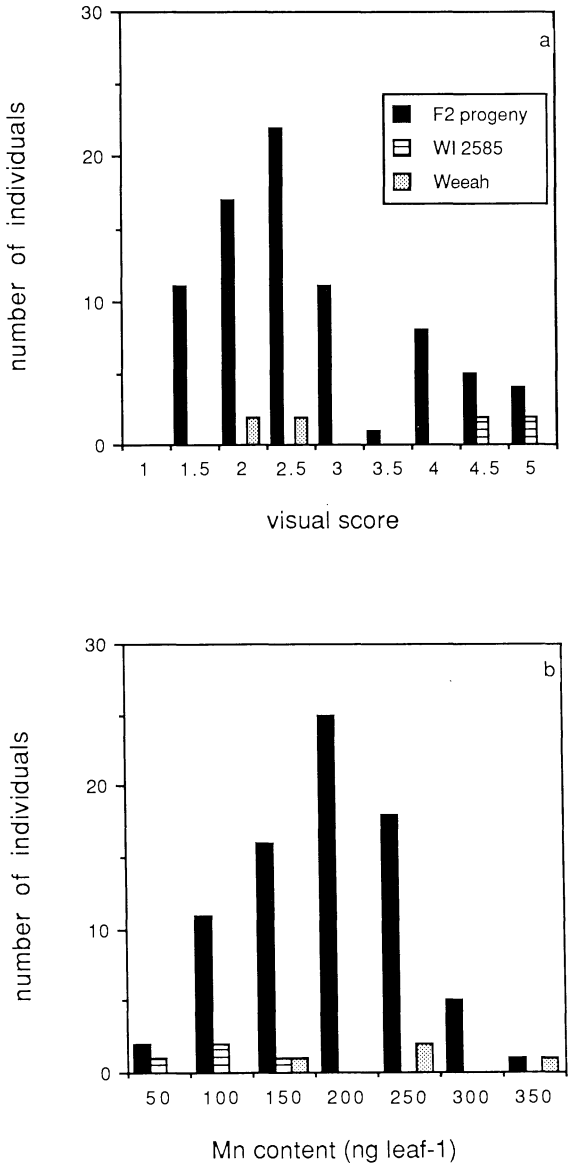

Fig. 5. Screening of the F_2 generation of a cross between the Mn-efficient Weeah and inefficient WI2585 based on visual scores (**a**) and on Mn content of YEBs (**b**). The number of individuals in each category is given.

most efficient genotypes must be separated from the intermediate and inefficient ones. The visual scores of plants in the growth cabinet separate the 2 most efficient lines in the field, WEE and WA, from the remaining lines (Fig. 2a). However leaf Mn and fluorescence do not separate efficient and inefficient barley lines (Figs. 2 and 5). Similarly, Marcar and Graham (1987) found that Mn-efficient and inefficient wheat genotypes could not be separated on the basis of leaf fluorescence and there were no differences between genotypes in shoot Mn concentrations in their nil Mn treatment. Preliminary experiments had shown no consistent differences between Mn-efficient and inefficient barley lines in the effect of Mn deficiency on tillering, height and leaf number. Thus, these were not measured in this experiment.

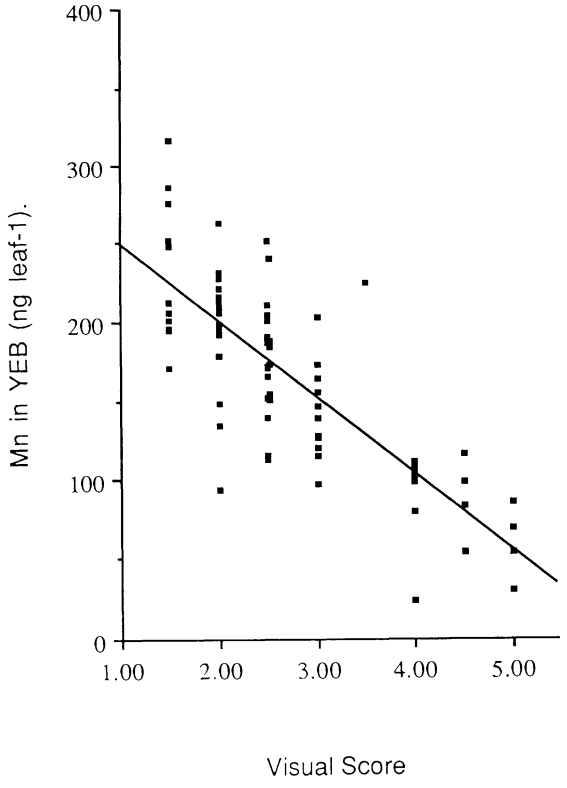

Fig. 6. Relationship between YEB Mn content (ng Mn leaf^{-1}) and visual scores. $R^2 = 0.64$.

While Mn content and concentration of the YEB and YEB + 1 and leaf fluorescence do not separate efficient from inefficient genotypes, they do correlate well with visual scores (Figs. 4 and 6). This can be explained by the big effect of Mn treatment on these measurements (Fig. 2b and 2c). Thus, while these 2 measurements do not separate genotypes, they are useful in diagnosing Mn deficiency. Mn-deficient leaves have much lower Mn concentrations and higher leaf fluorescence. However, the variability in these measurements makes them less useful than visual scores in separating genotypes. For example, a plant with a visual score of 2 can have the same YEB Mn content as a plant with a score of 4 (Figs. 4 and 6). In the screening experiment, visual scores were compared to Mn content (Fig. 5) in their separation of the efficient WEE and inefficient WI 2585 parents and in the F_2 generation. The average visual score of WEE was 2.25 with a % coefficient of variation (CV) of 12.8 while WI 2585 had an average score of 4.75 with CV of 6.1. While the Mn contents of the parents were markedly different, with values of 226 ng leaf^{-1} for WEE and 82 ng for WI 2585, they were also much more variable, with CV's of 34 and 33, respectively. Thus, the visual scores would seem to allow for a more precise analysis of the genetics of Mn efficiency. The statistical analysis of data in Figure 5a shows a three to one ratio of efficient to inefficient plants in the F_2, using a visual score of 3.5 to distinguish between them. If a three to one ratio exists in the Mn content of the F_2, it could easily be masked by the variability of this measurement. McCarthy *et al.* (in preparation) have also examined the F_1 and F_3 of that cross and Longnecker, Sparrow and Graham (unpublished data) have examined the F_1 and F_2 of a cross between WEE and GAL. These results indicate that Mn efficiency in WEE is controlled by a single, major, dominant gene.

That there are not genotypic differences in Mn concentrations of YEB, YEB + 1 or whole shoots while there are differences in visual scores of those same plants indicates that there may be differences in partitioning of Mn within the leaves so that more of the Mn in Mn-efficient shoots is physiologically available. Mn deficiency disrupts photosynthesis and has been shown to cause characteristic changes in chlorophyll a fluorescence in wheat (Kriedemann *et al.*, 1985) and barley (Hannam *et al.*, 1987). Thus, leaf fluorescence was examined as a measurement which could possibly separate Mn-efficient and inefficient genotypes. However, the lack of differences between genotypes in leaf fluorescence indicates that genotypic differences do not exist in photosynthetic capacity for a given amount of Mn in leaves. Also, preliminary results investigating water-soluble Mn in leaves from Mn-efficient and inefficient barley showed no differences between lines (Longnecker and Graham, unpublished data). Other investigations have found no differences between genotypes in critical Mn concentrations in barley (Harbard, 1988) and in wheat (Marcar, 1986). Possible mechanisms of Mn efficiency are discussed by Graham (1988). It may be that the main difference between Mn-efficient and inefficient barleys is in their uptake of Mn rather than in their Mn requirement.

In breeding soybeans for increased resistance to iron-deficiency induced chlorosis (IDC), it has been found that once populations are improved, it becomes increasingly difficult to separate genotypes

by visual scores. Piper *et al.* (1986) have found that stem cutoff increases the severity of Fe deficiency and allows for selection from improved populations. We have also found that cutting back barley plants to ground level increases the severity of Mn deficiency of the regrowth. Stem cutoff can also be used to minimize the effect of seed Mn on early plant growth (Longnecker and Graham, unpublished data, Uren *et al.*, 1988). Additionally, the most inefficient genotypes frequently do not regrow at all, which helps to eliminate plants from further development in the breeding program. On the other hand, this can be a problem in genetics studies in which an objective may be collection of seed of all genotypes, including inefficient ones. In studies such as the screening experiment described here, visual scores alone must be relied on for separating efficient and inefficient genotypes.

In summary, screening is currently based on visual scores and assessment of regrowth after stem cutoff which distinguish relatively large differences between efficient and inefficient genotypes. This is similar to breeding programs for IDC-resistance (Fe efficiency) in which visual scores of Fe-deficiency symptoms are used for screening (Fehr, 1982; Fairbanks *et al.*, 1987; Zaiter *et al.*, 1987). Other measurements such as Fe^{3+} reduction by soybean (*Glycine max* L.) roots (Tipton and Thowsen, 1983), release of root reductants by sorghum (*Sorghum bicolor* (L) Moench) (McKenzie *et al.*, 1985), and p-coumarate hydroxylase activity (an important enzyme in caffeic acid synthesis) in sorghum (McKenzie *et al.*, 1987) have not been successful in quantitatively separating IDC-resistant and susceptible genotypes. It is possible that future studies which examine phytosiderophore production could quantitatively separate IDC-resistant and susceptible genotypes in the Graminaceae (Longnecker, 1988).

It is difficult to explain why the visual scores of Mn-efficient and inefficient lines separate them better than their Mn content. As the barley lines in the breeding program improve in Mn efficiency, it is likely to become increasingly difficult to distinguish the most Mn-efficient lines. An understanding of the physiological mechanism(s) which confers Mn efficiency would perhaps provide a qualitative test for Mn efficiency, permitting much greater specificity in screening. The preliminary genetic evidence supports the hypothesis that a single physiological mechanism exists for Mn efficiency in barley.

Acknowledgements

The authors gratefully acknowledge the assistance of G Card with the growth cabinet experiments, C Reid, and I melky with analytical work, B Growden and R Bunder with the field experiment, Derm and Gwen Ottens on whose property the field experiment was conducted and the Barley Research Committee of South Australia who supported this work.

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Session 3

Screening techniques for detection of nutritional deficiencies and abiotic stress under genetic control

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 283–289.
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PLSO PN 166

Screening techniques for plant nutrient efficiency: Philosophy and methods

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Key words: Al- Mn- and heavy-metal-toxicity, Fe-deficiency, genotype, low-input, nitrogen utilization, nutrient efficiency, salinity tolerance, screening, selection

Abstract

A practical breeding and selection programme for a nutrient constraint situation requires that genotypes perform satisfactorily in appropriate soil conditions. Clearly if selection and variety development can be done in soil then the agronomic characteristics and performance can be assessed from the start. However, for a number of reasons this is not always practical and selection must be carried out in artificial systems. Such selection must be validated by soil trials.

Selection for toxicity tolerance, *e.g.* Al, Mn, NaCl and heavy metals is comparatively easy, but selection for tolerance to low levels of micronutrients seems considerably more difficult. Similarly the search for more fertilizer efficient and N-responsive cultivars, and the possibility of developing varieties for low-input systems still requires careful definition of the physiological ideotype and development of practical screening methods.

The development of screening techniques for these and other nutrient constraints is discussed.

The problem of screening

The newcomer to the field of genetic effects on plant nutrition might reasonably wonder why there is a whole major session devoted entirely to screening techniques, and why it should need an introduction. Surely, if there are significant differences they must be easily recognized? This is often true, but in practice the problems of soil and plant variability, and in the case of major nutrient efficiency defining the cultivar sought, are often quite difficult.

As one of the main purposes of investigating genetic effects in plant nutrition is to apply the results to develop improved or more appropriately adapted cultivars, this implies conscious selection and incorporation in a plant breeding programme. In parenthesis we may note here that the study of genetic variation in nutrition for its own sake is doing much to improve our knowledge of the mechanisms of plant nutrient uptake, transport and assimilation (Läuchli, 1976).

One of the reasons that plant breeders have, except in a few cases, avoided selection for specific nutritional response, is that it has hitherto had a rather low priority in all the factors that they have to consider, coming long after general selection for yield, quality, and disease resistance. Any plant selection programme requires the capacity to screen large numbers of plants in the early stages. In the case of nutritional characteristics this implies hundreds of plants, and possibly thousands. Therefore any screening and selection process must be basically straightforward and labour efficient (Gerloff, 1987; Graham, 1984; Horst, 1987; Shannon, 1984).

I take it as almost axiomatic that we seek tolerance or efficiency factors in the basic breeding material. Brady (1975) likened the development of an adapted variety to a rope in which the finished variety, represented by the rope, is built up from separate strands representing agronomic characters, disease and insect resistance, protein content and quality factors, and tolerance to adverse soils, low temperature, flooding *etc.* Which strand should be the central one? Obviously agronomic characters, deriving from existing material of known good yield potential are very important. However if a fundamental requirement

of the cultivar is that it should be adapted to very extreme conditions of exceptionally low pH soils or heavy metal toxicity or salinity, then probably the basic material must be chosen for this characteristic, as these qualities are rare in crop plants (Christiansen and Lewis, 1982; Jones and Qualset, 1984; Vose and Blixt, 1984; Wright, 1977).

Ideally, selection should be done in the field under conditions which will be characteristic for the variety being developed. In practice, finding suitable fields is often difficult, as the fields of research stations are almost universally of high fertility, and have had soil amendment practised for years. Moreover, varieties developed under such conditions may have significant defects when grown under farmers' situations. There are many examples: the garden beet variety 'Good for All' developed for canning turned out to be extremely susceptible to boron deficiency (Walker *et al.*, 1945); 'improved' soybean varieties developed Fe chlorosis on calcareous soils in Iowa because they had never been tested for Fe-deficiency tolerance (de Mooy, 1972); the oat variety 'Maelor' developed at Aberystwyth on relatively acid soils turned out to be particularly sensitive to Mn-deficiency (Vose and Griffiths, 1961). On a wider scale, the CYMMYT wheat varieties, characterized by 'Sonora', which has shown great adaptability in many parts of the World, is quite unsuited to the acid soils of Brazil and Central Africa. The Michigan family of Phaseolus varieties related to 'Sanilac' are similarly unsuited to acid soils. Clearly in these cases selection pressure has been zero for the defective character. Hopefully, as breeders appreciate the possibility of the unconscious incorporation of nutritional defects then wider testing in farmers' fields will take place earlier.

Although screening and selection under the appropriate field conditions might be the theoretical ideal, there are many compelling reasons that have led investigators to choose artificial systems. These include the inconvenience of distant test fields; the need to avoid site and seasonal variability; the need to screen very many acquisitions or selections of which there is too little seed available for field plots; the desirability of working during a longer season than is possible in the field; the need to keep costs down; the need to define more closely the selection environment; and the need to select for a character impractical in the field *e.g.* root morphology, rate of root growth, root excretions or reaction *etc.* (Horst, 1987; Vose, 1987).

Practical approaches to screening

Screening techniques can be considered collectively on a 'method' or 'problem' oriented basis. The latter is adopted here as being more generally appropriate.

Tolerance to Al- and Mn-toxicities

Together with Ca and P deficiencies, toxicity due to Al and Mn is the prime cause of crop failure on acid soils. Probably, tolerance to Al and Mn toxicities involves the easiest of all screening procedures. Thus in Brazil the soils are so extremely high in exchangeable Al that unamended soil provides a more than adequate screening material, with unadapted wheats barely emerging (Brown, 1977). In artificial systems the principal difficulty is judging initially the concentrations of Al or Mn in solution that permit differences in root or plant growth to be expressed, and guessing what increase in concentration is necessary for the next selection generation (Vose and Randall, 1962). Either too high or too low concentration will fail to select, the one because all genotypes will be affected, and the latter because the true degree of tolerance of tolerant genotypes will not be seen. Al presents a particular problem in that within certain limits seedlings can adapt in the short term to higher than normal Al (Vose and Randall, 1962). It is therefore essential that the chosen Al concentration is sufficiently high that it is beyond the adaptive range. Basically this depends on trial and error at first, as it depends on both the material and the level of the accompanying nutrients in solution.

Al-toxicity is shown quite clearly in short experiments even at the seedling stage, through lesser growth, paler green leaves and the characteristic reduced root growth, with 'stubby' secondaries. In contrast, Mn-toxicity usually takes much longer to develop and the effects are not so clear-cut. Frequently with grasses there is no diminution in growth even with quite high leaf-Mn levels and prominent brown speckling of the leaves. This makes scoring genotypes for Mn-tolerance much

harder and may require the plants to be grown to a much older stage.

While the screening of grasses and cereals for tolerance to Al-toxicity/low pH soils is straightforward, there is a more complex problem with dicotyledonous legumes such as Phaseolus. The requirement for Ca and P is much higher for these plants and most high Al soils are also low in available Ca and P. Does a Phaseolus variety which is tolerant to acid, high exchangeable-Al soils have tolerance to Al, to low P to low Ca or all three? (Vose, 1982). This is an open question which has significance for screening, because while screening in the appropriate soil will automatically balance these constraints, to achieve the same in an artificial system is clearly more difficult, although the artificial system will be more specific. Field conditions have been very successfully used to screen Al-tolerant from non-tolerant varieties (Foy *et al.*, 1965; da Silva, 1977; Spain, 1977) and offer the benefit of true agronomic yield and other data. The IRRI programme of screening the world rice collection for tolerance to acidity, alkalinity, salinity and Fe-toxicity and deficiency has made good use of modified small field plots, together with modified soils in tanks or flats, and such selections have been immediately available to the breeders (Ponnamperuma, 1977). Nevertheless, for selection of individuals at the seedling stage soils tend to be less useful. Moreover, the possible interactive effects in soil of Mn-toxicity and Mo, and Cu-deficiencies and different requirements for P can clearly confuse the situation in soil as it relates to Al tolerance or toxicity (Foy, 1977). Campbell and Lafever (1977) recorded that 'because of these complications (*i.e.* variability within and between years, need for replications, intensive labour requirement *etc.*) it was concluded that Al-tolerance can best be screened for in the laboratory with field observations to confirm that selected lines are indeed tolerant.'

Sand culture with nutrient solutions were used for some of the earliest screening work for Mn (Dessureau, 1958) and Al (Vose and Randall, 1962) tolerance, and subsequently many investigators have found good agreement between cultivars screened in nutrient solutions and appropriate soils (Campbell and Lafever, 1977; Howeler and Cadavid, 1976; Reid *et al.*, 1971). Maintaining pH control and a constant level of Al^{3+} in solution are important factors in nutrient solution screening techniques, and there have been different approaches to the problem.

It was found (Vose and Randall, 1962) and later confirmed (Foy, 1974) that cultivars which are adapted to acid soils have relatively low cation exchange capacity (CEC). This selection parameter cannot be used on the scale of individual plants because the method of determination requires too much material and is rather slow. However it could certainly be used for screening collections for cultivars tolerant to soil acidity. As the determination can be carried out on leaf material (which reflects root CEC) it is a method that could be applied to any varieties grown on trial plots without special conditions.

Tolerance to salinity

There are two major problems in selecting for salinity tolerance. The first is that tolerance to salinity varies with the age of the plant, and a plant which is tolerant at the seedling stage does not necessarily have the same tolerance when older. Secondly, there is more than one salinity factor, so that although popular thinking may regard NaCl tolerance as pre-eminent, in fact salinity may be due to chloride, sulphate and bicarbonate salts of calcium and magnesium as well as sodium (Epstein and Rains, 1987; Niemann and Shannon, 1977). Indeed there is a view which regards the suppression of plant growth by salinity as a non-specific effect that is related to the total concentration of salts in the root medium, which implies that salinity tolerance is purely an improved capacity for osmoregulation (Niemann and Shannon, 1977).

In fact there is some justification for concentrating on NaCl as the major problem, when one considers the extensive areas of coastal rice paddies and coastal irrigation aquifers subject to marine seepage. Moreover, in the case of NaCl tolerance we do have some knowledge of possible mechanisms, in addition to the generalized capacity to tolerate high osmotics. The unique objective of trying to select genotypes which can be irrigated by seawater (Epstein and Norlyn, 1977) also justifies the use of screening accessions in solution with NaCl as primary salinity, and a majority of the literature indicates that this is a generally accepted view.

Thus Ponnamperuma (1977) used soil in shallow trays salinized with common salt for large scale screening of rice varieties from the world collection, the plants being scored for dead leaves 4 weeks after transplanting. This method made possible the screening of some 4000 lines a year. In order to further test tolerant lines under field conditions of insects, disease *etc.* primary lines are further tested on field plots salinized with common salt to give an ECe of 8–10 mmhos/cm at 25°C. The ECe is measured during the test and if necessary more salt is added.

In contrast, Epstein's group (Epstein, 1977; Epstein and Rains, 1987) have used extensively large solution culture tanks in the greenhouse, salinized either with NaCl or a synthetic sea salt mix for initial selection. Selections have been subsequently field tested under irrigation with sea water at different concentrations.

Attempts have been made to select via callus culture mutant cell lines with high NaCl tolerance (Nabors and Dykes, 1985; Rains *et al.*, 1980; Ram and Nabors, 1986). As salinity tolerance is at least partly based on whole plant factors such as uptake and transport, it is difficult to see cell culture as a regular practical method of screening and selection. Undoubtedly enhanced salinity tolerant cell lines can be developed, but whether this is reflected in regenerated whole plants is another question. Indeed the question of regeneration is also a problem, as the longer a line is developed as callus the more difficult it is to regenerate. Another problem is obviously that of retention of useful agronomic characters in the regenerated plants. Undoubtedly plant tissue culture has a role, but we shouldn't expect it to be a universal solution.

Tolerance to nutrient deficiencies

The selection for tolerance to low available levels of iron and micronutrients seems to present more problems than selection of cultivars tolerant to toxicities. Although there are in the literature examples of varieties which seem to require lesser amounts of micronutrients for normal functioning, in general it is a question of more efficient acquisition whether through reactions at the root surface or root morphology. This is because in most areas of the world iron and microelement problems are not due to absolute deficiency, but are largely due to unavailability, as a consequence of soil pH, and possible competition from other elements. These effects are enhanced under drought conditions, which limit the release, movement and uptake of microelements. Thus in a dry year microelement observed deficiency may be severe, and yet not be noticeable in a year with adequate rainfall. For example, the author once attempted to screen for Mn-deficiency tolerance in oats, Avena, using trays of soil taken from a field which had plants with terminally severe Mn-deficiency symptoms. In the glasshouse, with necessary watering, no signs of Mn-deficiency appeared on even the most susceptible lines.

Thus the problems of field evaluation and selection are (1) year on year variability, and (2) spatial variability of soil within the field. Where field soils are relatively uniform and weather reasonably predictable then field selection for microelement efficiency is a possibility, as demonstrated by Graham (1984). Nevertheless, for the difficulties noted, plus cost, very many workers have preferred to develop glasshouse, growth room and indirect tests. Moreover, such tests permit the clear definition of the nutrient response for which selection is desired. Probably most workers have tried to avoid field procedures at an early stage of selection.

Randawa (1977) has described routine methods of screening for micronutrient deficiency susceptibility in India, in sand culture, pots and in the field, but noted that field tests are preferred and that pot tests may not indicate the same relative order of susceptibility. This is probably because a variety with a much branched root system but poor inherent micronutrient uptake capacity might appear much better in the field than in pots where root growth would be unimportant. Agarwala *et al.* (1971) used sand culture to screen a great number of crops for Zn, Mn, Cu, Mo and B deficiency susceptibility.

In the case of iron deficiency chlorosis, Brown (1977) has suggested initial selection in culture solution, growing plants at first with normal nutrient solution and then with a much reduced iron supply, when susceptible plants become chlorotic. Adding P to the solution increases Fe stress. Williams *et al.* (1987) have compared evaluation of sorghum populations in nutrient solution in the growth room and under field conditions. The

growth room experiments took 2 weeks and the field experiment 2 months, and both methods showed differential chlorosis between genotypes. The field experiment was confounded by drought and necessary irrigation. Correlation between field and growth room chlorosis rating was not very good, and it was concluded that experimental error should be reduced through additional replication or improved techniques before laboratory results can be applied to field research. Fehr (1985) had a similar problem with soybeans and resorted to field testing to differentiate soybean genotypes tolerant to iron chlorosis.

Iron absorption and transport is mediated by the release of hydrogen-ions by the root, thus lowering the pH of the root zone, favouring Fe^{3+} solubility and reduction of Fe^{3+} to Fe^{2+}. Laboratory methods have been now developed to quantify release of H^+ (Marschner *et al.*, 1982) and increase in reductive capacity (Marschner *et al.*, 1986). Potentially these methods should permit screening the capacity of genotypes to release nutrients such as Fe and Mn as shown for the soybean. Such methods will not of course tell us about root morphology (size and branching of root system) effects which may also be important. Nor will these methods take account of the plant internal factors which affect their resistance or susceptibility to Fe or Mn deficiency. Certainly with Mn these relate to different internal Mn pools (Munns *et al.*, 1963) and transport factors. For example certain Mn-deficiency tolerant oat varieties have more Mn in their roots than susceptible types (Vose and Griffiths, 1961) and excised leaf discs of tolerant types have greater capacity to take up ^{54}Mn from solution than have susceptible (Vose, 1962). Transport and leaf factors are also clearly a part of the problem in Fe chlorosis.

Screening efficient cultivars

The basic problems in screening for 'efficient' cultivars consist of definition and objectives. Different workers have defined efficiency in different ways, but the majority seem to favour a definition which relates to the units of dry matter produced per unit of the nutrient element under consideration. There is an adequate literature that attests to the capacity of certain genotypes to produce more per unit of nutrient than do the majority, due to unknown internal factors. Certainly the work of the Madison group has shown this clearly for P, K, N and Ca under controlled conditions (Gabelman and Gerloff, 1982; Gerloff, 1987).

However, when we consider the yield improvements in wheat and rice over the last 20 years it is appreciated that these have come about through selection for improved Harvest Index, *i.e.* dry matter has been moved from unwanted parts such as excess leaves and stem to the more desired grain component; there has not been much increase in total yield per unit of fertilizer. The efficiency of these varieties is apparently improved because less P and K is required to support unwanted leaves (Mengel, 1982). This has been discussed at length elsewhere (Vose, 1984), and has posed the question do low input cultivars necessarily imply low yield cultivars? Probably not, but it is true that wild plants which are perfectly adapted and persistent usually have low requirements for nutrients, but also have low yield potential (Chapin, 1980). This is in effect the background of landraces, which tend to have relatively low yield, require relatively low nutrients, but are also unresponsive to nutrients.

There are two potential end-users or more efficient cultivars: developed farmers who seek more efficient fertilizer use, greater economy, and less NO_3 pollution of groundwater, and farmers in developing countries who require varieties which can produce a reasonable yield with minimum fertilizer input. Possibly these two objectives are not incompatible in one respect: selection for larger and more branched root systems. This would be one approach to selecting cultivars which intercept N fertilizer more effectively, thus reducing groundwater contamination. It would also provide for low-input systems greater foraging ability of the plant, exploiting a larger volume of soil and enhanced input of P, Fe, Mn and Zn. Present day varieties are well able to produce photosynthates sufficient to support a larger root system.

There is adequate literature to show that there are N-responders and non-responders (Vose, 1987), and the second approach is clearly that of Gabelman and Gerloff (1982): seeking cultivars which produce the most dry matter when under restricted supply of N, P and K. Cultivars from this primary type of screening must then be taken to see how they perform in soil under realistic conditions

against standard varieties. This clearly is the difficult step, but here again simultaneous development of cultivars for high-input and low-input situations is not incompatible, because although modern cereal varieties perform optimally with high fertilizer, they are also quite efficient in their use of N, P and K.

Conclusions

The theme running through this paper is clearly that as yet there is no 'correct' method of screening for varietal differences in plant response to deficiency or toxicity, or major nutrients. Different methods have served equally well in the hands of different investigators. The key requirements are that they are efficient, simple and capable of identifying single tolerant/efficient plants.

The search for 'indirect' and laboratory methods which reflect field response will continue and should be helpful for characterizing individual plants for breeding programmes. However it is essential that all such methods, indeed any method, must be evaluated under field conditions, although the correlation between laboratory and greenhouse tests and field experience seems in general to have been quite good.

Where we still have difficulties is in the area of efficiency of nutrient utilization and the related search for low input varieties. A basic problem seems that we have not collectively defined what is being sought, and for what purpose. Is it to try and reduce fertilizer requirements for high-tech cultivation or is it to develop varieties that will produce reasonably under low input systems of small scale peasant farming? Maybe these two valid objectives require different approaches, but conceivably they could be the same.

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PLSO PN 167
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Biochemical techniques for genotype characterization

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Key words: barley, biochemical markers, electrophoresis, genotype identification, Jerusalem artichoke genotype, nitrogen efficiency, oats

Abstract

The techniques mainly used for genotype identification and characterization are presented and discussed in respect to their suitability and performance. Procedures are described for distinguishing between varieties and wild types of oats (*Avena* spp.) as well as varieties and provenances of Jerusalem artichoke (*Helianthus tuberosus* L.) using electrophoretic techniques. First experiences in looking for electrophoretical markers in respect to "low input" genotypes of barley, especially nitrogen efficiency, are presented and discussed.

Abbreviations. IEF: isoelectric focussing; rp-HPLC: reversed phase high performance liquid chromatography; RFLP: restriction fragment length polymorphism; SDS: sodium-dodecyl-sulphate; PAGE: polyacrylamide gel electrophoresis; Poro-PAGE; porosity gradient PAGE; PVPP: polyvinylpolypyrrolidone; PVP: polyvinylpyrrolidone; TRIS: α,α,α-Tris(hydroxymethyl)-methylamin

Introduction

Biochemical procedures for the identification of genotype differences are electrophoretic techniques, IEF, rp-HPLC, immunoelectrophoresis, and RFLP. Some of these methods have already been successfully applied for testing morphologically identical genotypes in respect to possible biochemical variation, that means for the demonstration of genetic variability. Therefore, it is helpful to employ some of these procedures additionally for detecting "low input"-genotypes early in the life of germinating seeds or seedlings. It is clear that a major advantage of biochemical methods in contrast to experiments in the field or greenhouse is the rapidity of the analysis that can be conducted on a large number of individual plants, often as seeds or seedlings.

First of all, Table 1 gives a general survey of the different biochemical techniques that are applicable in respect to genotype characterization.

The methods mainly used are electrophoretic techniques. By placing tissue extracts in a gel medium like starch, agarose or polyacrylamide and applying an electrical field, protein molecules migrate at a rate determined by their net charge and the pH of the medium. In addition, the network of gel molecules sieves migrating molecules according to their size and shape.

One possibility is the examination of storage proteins located in the endosperm of cereal grains that function as nitrogen reserve. They are composed of prolamines and glutelins.

A second group of proteins can be summarized as functional proteins that can be separated in

Table 1. Techniques of genotype characterisation

1)	Electrophoresis	— PAGE ⟶ proteins, esterases
		— SDS-PAGE ⟶ peptides
		— Poro-PAGE ⟶ peptides
		— 2-D-PAGE ⟶ proteins, peptides
		— SGE ⟶ isozymes
2)	IEF (isoelectric focussing)	
3)	reversed phase HPLC	
4)	RFLP (restriction fragment length polymorphism)	

respect to their origin being the endosperm or embryo. All of them have specific physiological functions as enzymes, activators or inhibitors.

The advantages of working with storage proteins are:
— easier detection
— broader genetically determined variability
— higher independence from external growth factors

Storage proteins can very easily be detected by unspecific protein dyes. Enzymes can also be separated into discrete bands, but their position has to be visualized by the use of specific stains. A disadvantage of using the storage proteins is that they are coded only by a few chromosomes of the whole genome. In wheat for instance only two of seven chromosomes are involved (Ohms, 1988).

Electrophoretical techniques have been used in several tissues including seeds, leaves, roots, bark, fruits, and pollen; the material must be fresh or frozen and sometimes the extract has to be dialysed prior to electrophoresis in order to remove salts or complexing substances. The usual procedure of PAGE is roughly described in Table 2.

Table 2. PAGE-Electrophoresis

Crushing of single seeds
↓
Adding of extraction medium, incubation
↓
Centrifugation (18000 × *g*)
- -
Preparation of the gel
↓
Removing the comb, filling the electrophoretic tanks
↓
Loading the samples
↓
Applying the suitable constant voltage
↓
Fixing and staining the gel
(perhaps destaining afterwards)
↓
Documentation and storing the gels

Discontinuous systems are useful when greater volumes must be applied; a sharpening of protein bands during electrophoresis can be achieved by combination of a stacking gel with a separation gel, possibly interrupted by a spacer gel. This positive effect is mostly pronounced in the upper gel part. SDS-PAGE is the abbreviation for the use of sodium-dodecyl-sulphate, an anionic detergent, which converts native proteins into a "random coil"-structure with a simultaneously loading these peptides with SDS. That means, it unfolds proteins and applied mercaptoethanol opens the disulfide bonds. Poro-PAGE stands for the application of polyacrylamidegels with increasing acrylamide-concentration. This variant also allows the determination of the molecular weight of native proteins.

Isoelectric focussing (IEF) is a technique for the separation of proteins exclusively according to their charge. It uses the different net charge of proteins for their separation in a pH-gradient. These gels are generally only about 0.2 mm thick and have to be handled by using a carrier foil. This method achieves a high resolution and involves only quite low costs. Staining and drying is very fast. A disadvantage is the high number of bands, which make the interpretation difficult and time-consuming.

Two-dimensional-(2D)-electrophoresis is the combination of a PAGE-technique in one and IEF in the other dimension. It is also known as "mapping". It is very time-consuming and difficult in interpretation and therefore not applicable as a routine technique. However, very often one of these methods is not sufficient for the desired topic. For genotype identification, normally several PAGE-procedures and IEF are combined (Ohms, 1988).

An alternative to the examination of total protein patterns is the analysis of isozymes obtained by replacing the general protein stain by specific enzyme stains. In this case, a suitable extraction medium is of particular importance to protect enzymes against the adverse effects of certain cell constituents which are released on maceration. Some typical additives to such an extraction medium are shown in Table 3.

An alternative to PAGE is starch gel electrophoresis. This technique is used for isozyme characterization, since it bears the advantage, that the gel can be sliced into several gel slabs, which can

Table 3. Components of extraction media

1) Reducing agents (ascorbic acid, potassium metabisulphate, cysteine, mercaptoethanol, dithiothreitol) — avoiding of protein denaturation by the oxydative products of phenols
2) Polyvinylpyrrolidone, polyvinylpolypyrrolidone — removing of phenols from extracts by inclusion
3) Sucrose — inhibition of protein denaturation — enhancing of chloroplast disruption
4) Detergents (Triton X-100, Tween-20, Tween-80) — increasing the solubility of some proteins
5) Salts – increasing the ionic strength of the medium – providing enzyme cofactors

be stained for different enzyme systems. The disadvantage is, that in most cases the clarity of banding pattern is not very good.

By selection of appropriate conditions all these points are fulfilled (*e.g.*: growing plants in controlled conditions, taking comparable developmental stages *etc.*).

A number of enzyme systems can be used for genotype characterization (Table 4).

More recently, techniques of genotype characterization such as reversed phase HPLC (rp-HPLC) and restriction fragment length polymorphism (RFLP) have emerged. rp-HPLC uses the hydrophobic properties of soluble storage proteins for their separation and characterization. The protein profiles of different genotypes are compared by chromatogram overlay, difference plots between chromatograms, multivariate analysis, and cluster analysis. So rp-HPLC complements other procedures and has advantages in speed, sensitivity, resolution, quantification, and automation.

The most recent method is RFLP. Genomic diversity is assessed by fragmenting purified DNA with restriction endonucleases, separating the fragments by electrophoresis in agarose, and staining with ethidium bromide or novel techniques without the need of labelling. Several hundred restriction enzymes are known and available; each of them cuts DNA at a different position and hence produces a different pattern.

Materials and methods

A) Plant material and equipment

Barley and oats seeds as well as Jerusalem artichoke tubers used in these experiments represent material of the plant genetic resources collection located at the Institute of Crop Science and Plant Breeding of the Federal Research Centre for Agriculture (FAL).

The electrophoretical equipment was delivered by Biometra, the power supply from Pharmacia-LKB.

B) Protein extraction

Ten g of seeds were ground with a Pulverisette (Fritsch) using a 0.5-mm mesh sieve, washed twice with acetone and dried. 0.5 g of this washed flour was extracted with 3 mL of a solution containing 20% 2-chloroethanol, 3 *M* urea and 1% mercaptoethanol for at least 1 hour. After centrifugation (15 min 15,000 *g*) the supernatant was decanted and stored until use at 2°C.

Table 4. Enzyme systems applied for biochemical characterization

Aconitases	Glycerate-2-dehydrogenases
Alcohol dehydrogenases	α-Glycerophosphate dehydrogenases
Aldolases	Isocitric dehydrogenases
Alanine amino peptidases	Malate dehydrogenases
Malic enzymes	Malic enzymes
Endopeptidases	Menadione reductases
Esterases	Peroxidases
Glucose-6-phosphate-dehydrogenase	6-Phosphogluconate dehydrogenases
	Phosphoglucose isomerases
Glutamate-oxaloacetate-transaminases	Phosphoglucomutases
	Shikimic dehydrogenases
Glutamate-pyruvate-transaminases	Tetrazolium oxidases

Single seeds were squeezed with an electronic pliers and filled into an Eppendorff tube. After adding 300 μL of extraction medium (Marmulla, 1988), the samples were suspended with a Vortex mixer and incubated over night at 4°C.

Fifty mg leaves of 7–10 days old seedlings were homogenized with a mortar in 100 μL extraction medium containing 60 mg TRIS, 88 mg ascorbic acid, 3.7 mg KCl, 0.5 mg $MgCl_2$, 0.5 g PVPP, 0.25 g PVP-40 and 50 μL mercaptoethanol in 100 mL, filtered through a piece of lens paper and loaded into paper wicks (2.5 × 12 mm).

Frozen tubers of *Helianthus tuberosus* L. were homogenized and the homogenate squeezed through two layers of filter paper. The filtrate was centrifuged and directly taken for electrophoresis or stored at − 20°C until use.

C) Electrophoretic procedure

a) Polyacrylamide gel electrophoresis (PAGE). 75 mg sucrose were added to 0.5 ml protein extract of flour; front marker was 0.5% amido black or 0.25% methylgreen, respectively (Cooke and Draper, 1986). Protein extracts of single seeds were supplied with 45 mg sucrose.

Seven μL of each protein extract was loaded on top of the gel. Several acrylamide concentrations were tested (6–14%). PAGE at pH 8.9 was conducted with 125 m*M* TRIS/Borat buffer, PAGE at pH 3.1 in 8.5 m*M* aluminium lactate buffer with a tank buffer containing 4.25 m*M* sodium hydroxide, adjusted to pH 3.1 with lactic acid. Electrophoresis was carried out at 250 V for about 1 or 2 hours, respectively.

Gels were fixed and stained in acetic acid:methanol:water (1:6:14, v/v/v) containing 1% Coomassie Blue R-250. Staining was finished within 12–24 hours and, after washing in the same solution without Coomassie Blue, the gels were stored in polythene bags at 2°C.

b) Starch gel electrophoresis. A 12% starch gel was prepared according to Shields *et al.* (1983). The gel was cut into two parts (1/3 to 2/3) and the paper wicks, containing the samples, placed between them. Marking the front was performed with 0.5% bromophenoleblue. After loading the extract from the wicks to the gel by applying 150 V for a period of 20 min, the wicks were removed and the electrophoresis proceeded at 300 V, until the front had migrated to about 8 cm. The gel was slices, the upper and lower slice discarded, the others stained for specific enzyme systems.

c) Activity staining. Staining for esterase activity was performed in a freshly prepared mixture of 40 mg α-naphthylacetate and 100 mg Fast Blue RR-salt in 200 mL 0.3 *M* phosphate buffer pH 7.0.

Peroxidase activity was detected with a fresh prepared mixture of 0.05 g 3-amino-9-ethylcarbamate, 5 mL antipyridine, 0.5 mL 3% H_2O_2, 2 mL 1% $CaCl_2$ and 95 mL 0.05 *M* acetate buffer pH 5.0.

Results and discussion

Identification of varieties and genotypes of oats and Jerusalem artichoke

A part of the *Avena* spp. provenances present in the plant genetic resources collection have been screened in respect to genetic variability. First of all, it was of interest to investigate the percentage of duplicates in this collection. To start this, several electrophoretical techniques have been tested for their suitability for this purpose. Starch gel electrophoresis was applied to leaf extracts of young seedlings and several isozyme banding patterns were analyzed. The results are shown exemplary in Figure 1a and b.

In most cases, a clear distinction between the different species or provenances could be detected by evaluating esterase and peroxidase spectra. In these experiments, similar developmental stages were compared and not seedlings of the same germination period. However, the variation between seedlings of the same seed sample, that present a nearly identical growth stage, is not neglectible. Furthermore, some of the provenances do not represent a single genotype, so that the evaluation is quite complicated (Rühl and Standke, 1989).

Due to these problems, PAGE-techniques were applied to wild oats seeds. The starting point was an investigation of the best suited extraction medium. The most promising results were obtained using 25% 2-chloroethanol for protein extraction. In consensus with Cooke and Draper (1986), 12%

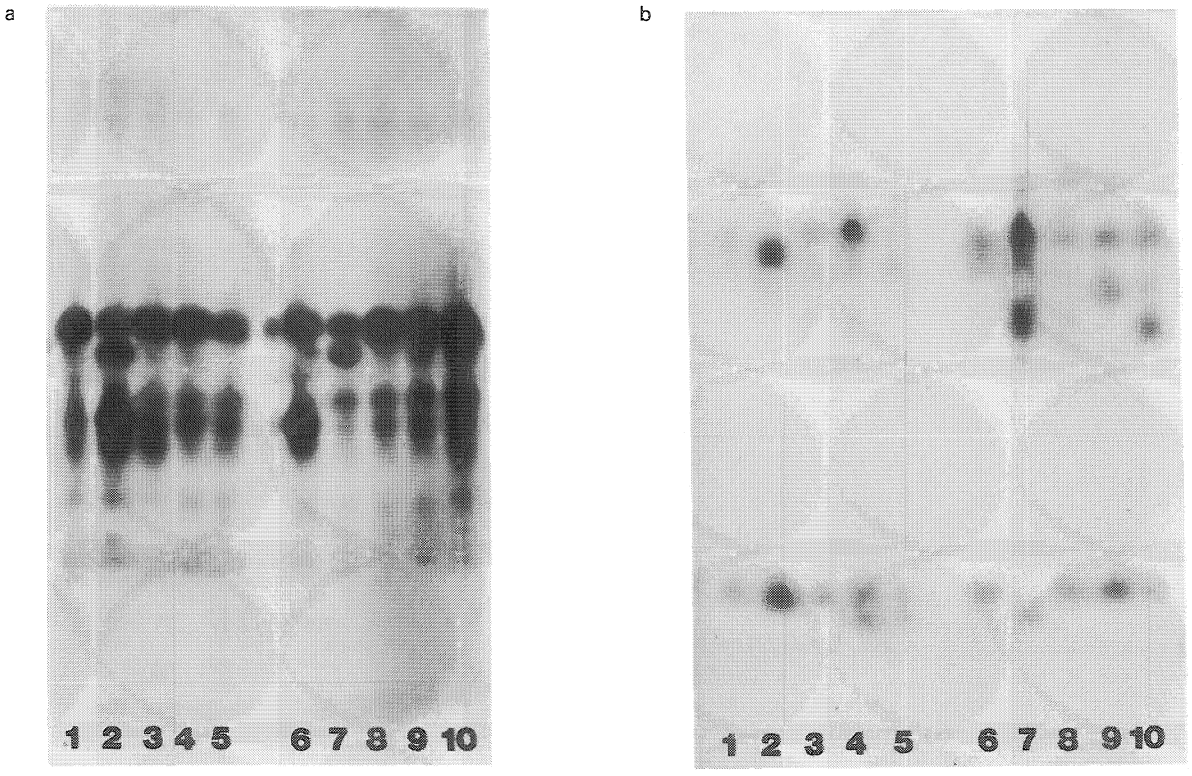

Fig. 1. Esterase (**a**) and peroxidase (**b**) banding pattern of different Avena genotypes separated by starch gel electrophoresis/ The tested genotypes were: 1) Rotenburger Schwarzhafer (*A. sativa* var. *nigra*); 2) Bensings Findling (*A. sativa* convar. *orientalis*); 3) Milton A (*A. sativa* var. *aurea*); 4) Nürnberger Hafer (*A. sativa* var. *mutica*); 5) Belem (*A. sativa* var. *chinensis*); 6) Grey Winter (*A. byzantina*); 7) Grey Winter × Kyko (*A. sativa*); 8) Grey Winter × Marvellos (*A. Sativa*); 9) black oats (*A. strigosa*, diploid); 10) wild oats (*A. sterilis*, hexaploid). Electrophoresis has been carried out as described above.

acrylamide led to the best resolution in banding pattern. Figure 2 shows an example of different oats genotypes that gave reproducible electropherograms.

The combination of this procedure with the evaluation of the esterase banding pattern led to a good ability to make a decision between the provenances tested (Rühl and Standke, 1989).

A second approach deals with the genetic variability of Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars and provenances. At time, the collection consists of 73 samples. By means of agronomical and morphological characters, it is not possible to distinguish between more than 20 different phenotypes. Therefore, electrophoretical techniques were applied to test the genetic diversity of this Jerusalem artichoke collection. Tubers were collected and an extract prepared as described above.

Since tubers of *Helianthus tuberosus* exhibit a dormancy period of more than 100 days, no significant changes should appear during the first three month after harvesting. However, preliminary experiments demonstrated changes in isozyme banding pattern even during this time of "dormancy"; so tubers had to be collected very soon after harvesting and tubers, as well as the tuber extract were stored frozen at a temperature of $-20°C$ until the date of evaluation (Standke, 1989). The best techniques for genotype identification in this case was the esterase banding pattern in porosity gradient gels like 10–28% acrylamide (Fig. 3). This procedure allows the differentiation between 49 different genotypes, that means nearly 2.5 × the visually distinguished phenotypes. A further method may be the evaluation of protein spectra, but the differences are not as significant as in the former case.

These findings are partly in consensus with results of Barloy (1988), but he looked at leaf

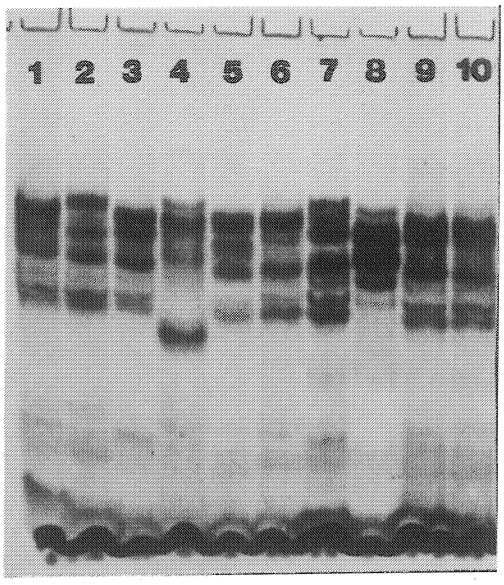

Fig. 2. Proteinograms of different genotypes of oats separated by PAGE at pH 3.1. The tested genotypes were: 1) Krafts Rheinischer Gelb (*A. sat.* var. *aurea*); 2) Mahndorfer Gelb (*A. sat.* var. *aurea*); 3) von Kalbens Vienauer (*A. sat.* var. *mutica*); 4) Peragis Früh II (*A. sat.* var. *aurea*); 5) Lischower Frühhafer (*A. sat.* var. *mutica*); 6) Wadsacks Gelb (*A. sat.* var. *aurea*); 7) Mauerner Weiss (*A. sat.* var. *Mutica*); 8) Weihenstephaner A (*A. sat.* var. *montana*); 9) NPZ Weiss (*A. sat.* var. *mutica*); 10) Peragis Weiss (*A. sat.* var. *mutica*). Protein extraction and electrophoretic procedure are described above.

esterase spectra by PAGE without porosity gradient and could not achieve a well pronounced banding pattern. Additionally, proteinograms only allowed to separate the seven investigated cultivars

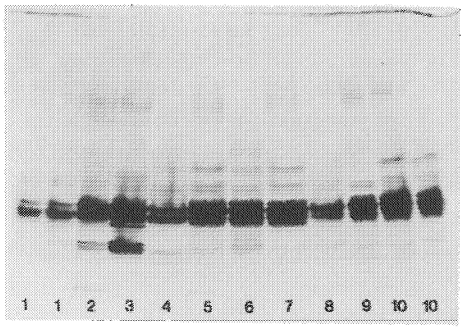

Fig. 3. Esterase spectra of 10 *Helianthus tuberosus* L. cultivars in a porosity gradient gel with 10 to 28% acrylamide. Exemplary shown are the results of the ten provenances tested first: 1) variety Bianca; 2) variety Waldspindel; 3) S3 (origin: Bavaria); 4) S4 (origin: Schleswig-Holstein); 5) S5 (origin unknown); 6) variety Rote Zonenkugel; 7) (origin: black forrest); 8) variety Medius; 9) variety Novost (USSR); 10) S10 (origin: black forrest).

into three groups. So here an improved system for the differentiation between Jerusalem artichoke cultivars and provenances is offered, solely basing on the evaluation of tuber extract, which is of great value for distinguishing between this material that apparently shows only minor genetical differences.

Biochemical markers of low input genotypes of barley

It is well known and accepted, that bread-making quality of wheat is correlated with certain seed storage protein subunits (Payne *et al.*, 1979). Furthermore, resistance of barley cultivars to some races of powdery mildew and the polypeptide composition of a storage protein (hordein) as revealed by electrophoresis are genetically linked (Riggs *et al.*, 1983).

So a first step in searching for protein markers

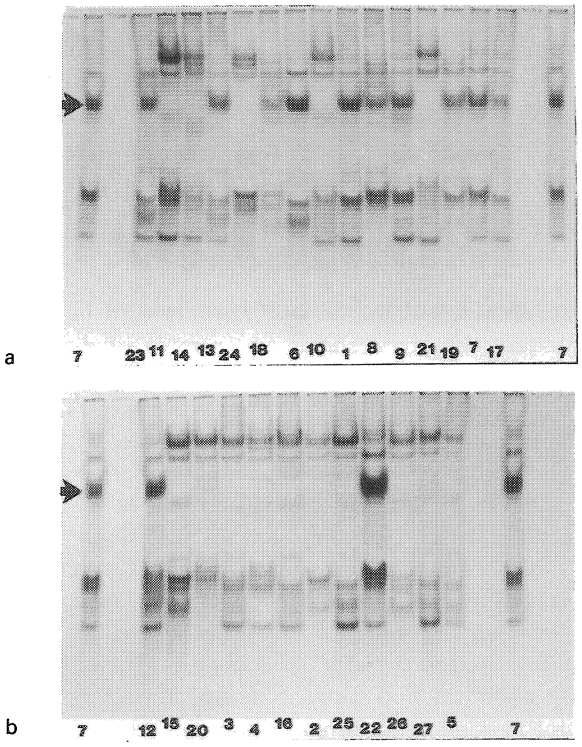

Fig. 4a and b. Protein banding pattern of barley genotypes differing in respect to nitrogen efficiency. The genotypes for this experiment were chosen according to the evaluation carried out by Dambroth and El Bassam (1987). Nitrogen efficiency increases from the samples loaded onto the left side of the gel to those of the right side and from gel a to gel b. PAGE was carried out with an acrylamide concentration of 10% at a pH of 8.9.

for low input genotypes was done by selecting appropriate electrophoretic procedures for separating the storage proteins of 27 barley genotypes, differing in respect to nitrogen efficiency, based on an investigation of Dambroth and El Bassam (1987). Several different extraction media were tested (Marmulla, 1988). The best results were achieved with 30% 2-chloroethanol as well as 6% urea. Extraction with 2-chloroethanol yielded a lower number, but clearer bands compared with 6% urea and 1% mercaptoethanol. In general, electropherograms of single seeds were more definite than those of flour samples. In the range of 6–12% acrylamide concentration, Disc-PAGE with 10% acrylamide produced the best resolution of proteins (Marmulla, 1988). Figure 4a and b show the storage protein banding pattern of the 27 tested barley genotypes. The sequence of the samples was chosen according to Dambroth and El Bassam (1987). Nitrogen efficiency increases from the left side of the gel to the samples on the right side of the gel, sample 7 served as standard.

These protein spectra do not show a significant peptide or polypeptide, that could be termed as a marker for the character "nitrogen efficiency". The band marked with the arrow is found quite often in genotypes with a low level of nitrogen efficiency (Fig. 4a) and is very seldom visible in samples of low input genotypes (Fig. 4b), but quite a lot of runaways exist.

SDS-PAGE (spectra not shown) led to a considerably increased number of bands, but clearer results could not be obtained (Marmulla, 1988).

Using specific enzyme stains for esterase and peroxidase isozymes served as next step. Indeed, esterase spectra of single barley seeds show a high degree of variation (Figure 5a and b), but no characteristic band for an easy biochemical detection of genotypes showing a high level of nitrogen efficiency could be seen. Similar results were obtained using leaf extracts in spite of single seeds (Marmulla, 1988).

The task of correlating physiologically important or desirable traits with electrophoretically obtained marker bands or spots is quite controversially discussed. Ohms and Paradies (1984) determined the bread-making quality of wheat by SDS-PAGE of seed glutelins. They detected a definite correlation between characteristic protein banding patterns and accompanying bread-making

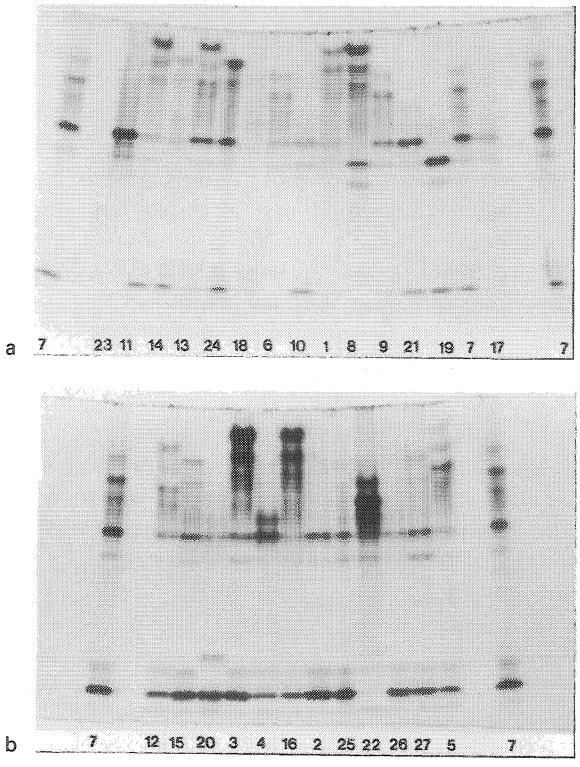

Fig. 5a and b. Endosperm esterase spectra of barley genotypes differing in respect to nitrogen efficiency. The genotypes for this experiment were chosen according to the evaluation carried out by Dambroth and El Bassam (1987). Nitrogen efficiency increases from the samples loaded onto the left side of a gel to those of the right side and from gel a to gel b. Disc-PAGE was carried out with am acrylamide concentration of 10% at a pH of 8.9.

quality; a change of banding behaviour was strongly correlated with a shift to decreased baking quality.

The same authors identified varieties of winter barley resistant to yellow mosaic virus by PAGE and IEF of the prolamine and albumin/globulin fraction (Ohms and Paradies, 1985). However, an association of this trait with one specific protein band was not possible. The identification of resistant varieties was carried out by means of the total variety specific banding pattern of these two protein fractions.

Hurkman and Tanaka (1987) investigated the effect of salt stress on protein synthesis in roots of barley seedlings by 2D-electrophoresis. They detected two proteins synthesized specifically in seedlings cultivated in salt solutions. Obviously, the expression of the corresponding genes was respon-

sible for the salt tolerance of these barley seedlings.

This trait as well as several resistances against mildew and rust, that have been looked at by Shewry *et al.* (1979), should also be of interest for the characterization of low input genotypes. However, also in this respect no single protein band was found, that could be correlated with these resistances.

Since nitrogen efficiency is a trait of complex physiological causes, we are dealing with a continuous transition and can not expect the appearance or disappearance of a single protein band. An increase of one or more distinct bands is certainly more likely.

A significant demand for these investigations is the still lacking knowledge about the genetic base of low input traits. A very important prerequisite is the identification of the loci on the corresponding chromosomes.

Further steps in searching for biochemical markers for barley genotypes showing a high nitrogen efficiency will be the evaluation of isozyme spectra of glutamate dehydrogenase (GDH) and glutamate-oxaloacetate-transaminase (GOT), since these enzyme systems should be important for the selected character "nitrogen efficiency". Preliminary experiments using crude leaf extracts were not very promising (Marmulla, 1988). ISo it seems likely, that mitochondria must be isolated prior to enzyme extraction in order to achieve a sufficient enzyme purity and yield (Yue, 1969).

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PLSO PN 168

Comparison of nitrogen utilization of diploid and tetraploid perennial ryegrass genotypes using a hydroponic system

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Key words: breeding, *Lolium perenne*, nitrogen, nutrient solution, ploidy level, ryegrass

Abstract

In the present study, genetic variation in growth rate of perennial ryegrass (*Lolium perenne* L.) was evaluated on a nutrient flow system (hydroponics) with computer controlled supply of nitrogen. The control algorithms were based on a simulation model designed to achieve a nitrogen supply proportional to growth. The setpoints of the growth rates, governed by limiting nitrogen supplies, were 20%, 50% and 100% of the optimal growth rate. Twelve tetraploid and 12 diploid cultivars were tested in a split plot design with 14 replicates. The leaf area indices at four successive harvests predicted by the model corresponded with the measurements within 15% deviation margins. The tetraploid cultivars yielded more at the first harvest but this trend was reversed at the fourth harvest, owing to a lower tillering rate in the tetraploids. Growth analyses at the different N-supply rates led to the following conclusions:

— A low N-supply leads to a low tillering rate. The decline is most manifest at organic N-contents below 2%. At comparable organic N-contents, the number of tillers of the tetraploids was smaller than that of the diploids. At the fourth harvest, the average tiller density of the tetraploid cultivars was reduced to 60% of the diploid tiller density.

— The leaf weight ratio (LWR) increased linearly with the organic N-content of the leaves. The effect was equal for diploid and tetraploid cultivars. The LWR increased from 0.40 at 1.5% N to 0.80 at 3.5% N.

— Specific leaf area (SLA) decreased with a decreasing N-content. The SLA of tetraploid cultivars was 10%–15% lower than that of diploid cultivars.

— Photosynthesis was linearly related to the organic N-content. The photosynthesis of the diploid cultivar Wendy did not differ from that of the tetraploid cultivar Condesa. However, a test of 6 tetraploid and 6 diploid perennial ryegrass cultivars under optimal N-supply revealed that the light-saturated net photosynthesis of tetraploid cultivars on a leaf area basis was 30% higher than that of diploid cultivars.

Introduction

Breeding of tetraploid cultivars of perennial ryegrass for grazing has been stimulated by their high digestibility and by the preference of grazing cattle for tetraploid cultivars. The lower tillering rate of tetraploid cultivars however, may lead to open stands and a low productivity of permanent swards. The tillering rate of grasses is determined by the rate of leaf appearance and the supply of assimilates available for the emergence of axillary tillers from the buds in the newly formed leaf axils. The theoretical maximum number of tillers appearing per tiller during a single leaf appearance interval (site filling) is 0.693 (Neuteboom and Lantinga, 1988). Suboptimal growth conditions may lead to decrease of the site filling or an increase of the leaf appearance intervals.

The interaction between morphological and physiological characteristics of a sward under a

regime of frequent defoliation is complicated. During a period of 4 days after cutting, the total weight of the sward remains approximately constant or slightly decreases and the expansion of new leaves is preserved from non-structural carbohydrates in the stubble (Alberda, 1966). The leaf area that is produced in that stage determines the light interception capacity at the start of the subsequent exponential growth and is therefore of crucial importance, but still received little attention in breeding programs. This initial leaf area is dependent on the amount of stored carbohydrates and the number of tillers (Alberda, 1966). In the subsequent exponential stage and during the transition to linear growth, the dry matter production is determined by the photosynthetic rate and by morphological attributes like leaf thickness and assimilate distribution.

In the present study, the response of tetraploid and diploid cultivars of perennial ryegrass to nitrogen is studied on a nutrient flow system (hydroponics). The response of both ploidy levels is compared by relating their growth parameters to the organic N-content of the shoots.

Genetic variation in nitrogen utilization may be worthwhile to exploit in breeding programs for low input cultivars. Information on this variation is also needed to develop controlled nitrogen management systems.

Material and methods

Seeds of 12 diploid and 12 tetraploid cultivars were sown in small pots in the greenhouse and after germination transplanted in open ended tubes filled with rockwool on 15 September 1987. The lower tube edges were in contact with a flowing nutrient solution (Steiner, 1979). Three series of 14 containers with a capacity of 25 L each were connected in parallel with 4 main reservoirs. In each series seven randomized containers were used for each ploidy level and on each container were two grids of 12 completely randomized plants of the respective cultivars. Adjustable aluminium screens around each container guaranteed a homogeneous light distribution. The average day and night temperatures were 17.3/13.3°C for the air and 15.8/15.0°C for the nutrient solution, respectively. High-pressure sodium lamps gave additional light over a period of 15.7 hours. The average photosynthetically active radiation at the top of the swards was 1.65 MJ m^{-2} day^{-1}. The pH of the nutrient solution was automatically adjusted to a value of 5.6.

The series corresponded with the treatments consisting of three N-application levels. The nitrogen supply was controlled by a simple simulation model that predicted the actual growth rate from intercepted radiation on a minutes time base.

$$I_{abs} = I_0\{1 - \exp(-k\ LAI)\}(1 - 0.08) \quad (1)$$

I_{abs} = absorbed photosynthetically active radiation (PAR, MJ m^{-2} day^{-1}), I_0 = incident photosynthetically active radiation at the top of the swards,
k = extinction coefficient (0.55),
0.08 = reflection coefficient of the canopy.
LAI = leaf area index ($m^2.m^{-2}$).

It was assumed that the relative growth rate was proportional to the organic N-content of the leaves (Agren and Ingestad, 1987; Hirose, 1988; Ingestad, 1982). Three levels of nitrogen were applied, either sufficient for the calculated potential growth rate or causing fixed reductions of 50% and 80%. At any time, the amount of nitrogen available was in equilibrium with the actual growth. The optimal N-application (100% treatment) was adjusted to give a total-N percentage of 4.7 in the leaves.

$$dLAI/dt = Ew \cdot I_{abs} \cdot SLA \quad (2)$$

SLA = specific leaf area of newly formed leaves (m^2 kg^{-1}).

E = shoot dry matter production per unit of absorbed PAR (g d.m./MJ). E was estimated to be 4.50 g MJ^{-1} for the 100% treatments (Schapendonk and de Vos, 1988), 2.25 g MJ^{-1} for the 50% treatment and 0.90 g MJ^{-1} for the 20% treatment, respectively. Thus growth was assumed to be proportional to the amount of nitrogen in the shoot and it was limited by balanced reductions of the relative nitrogen addition rate.

Shoots and roots of individual plants were harvested 4 times on 13 October, 11 November, 10 December 1987 and 12 January 1988. Dry matter of shoot and roots was determined after oven drying at 80°C. The total amount of nitrogen was determined according to method 2.058 and NO_3 according to method 7.041 (Official Methods to Analysis of the Association of Official Chemists, 13th edition, 1980, Washington DC). The latter was slightly modified adopting a continuous flow technique. For this experiment the SLA in the

control algorithm was estimated to be $40\,m^2\,kg^{-1}$ independently of the N-application. The actual SLA was determined at each harvest from measurements of length and width and the dry weight of the longest fully expanded leaves of each plant. In each treatment, light-saturated photosynthesis was measured on 14 plants of the cultivars Wendy and Condesa. Young fully expanded leaves were cut two days before each harvest and measured within 2 minutes after cutting. In a separate pilot experiment light-saturated photosynthesis of leaves from 6 randomly chosen diploid and tetraploid cultivars was measured in 4 replicates, at time intervals of 3 days. Gas exchange measurements were carried out with a portable leaf chamber analyzer (LCA, Analytical Development Co. (ADC), UK). Air was obtained from a gas cylinder to ensure constant composition. It was led over a water bath to humidify it before entering the leaf chamber. All measurements were done at light saturation, realized by an incandescent lamp cooled by a fan. Average conditions within the chamber were: $2000\,mol\,m^{-2}.s^{-1}$ ($340\,W\,m^{-2}$) of photosynthetically active radiation (400–700 nm), temperature of 24.6°C, vapour pressure deficit of 7.2 mbar, and CO_2 concentration of 320 bar. The number of live tillers was determined 1 week after the harvests.

Results and discussion

Tillering rate

The tillering rate depends on the assimilate supply and the number of axillary buds available, the latter being a function of the leaf appearance rate. Figure 1 shows the cumulated number of tillers of the diploid cultivars at subsequent harvests and different N-treatments (Fig. 1a) and the relative tillering rates for the respective harvests (Fig. 1b). In all treatments, the tillering rate of diploid cultivars exceeded that of the tetraploid cultivars, but the relative differences remained approximately constant at different N-levels (Fig. 1b). A lower N-application inhibited tillering, which was due to a lower site filling at the lower N-levels (Neuteboom *et al.*, 1988). Fig 1c depicts the tiller density of the tetraploids relative to the diploids, averaged over all N-treatments. From the first to the fourth harvest the ratio decreased from 0.82 to 0.60. Neuteboom *et al.* (1988) showed that the theoretical maximum number of tillers appearing per tiller during a single leaf appearance interval (site filling) was 0.693 for ryegrass. In the experiment described here, the greater number of tillers of cv. Wendy (diploid) compared to cv. Condesa (tetraploid) was not due to a difference in site filling but to different leaf appearance rates, which were 0.126 and 0.112 leaves/tiller/day at the 100% N-supply for Wendy and Condesa, respectively. The number of leaves at the first harvest of the diploid cultivars was on the average 14% higher than that of tetraploid cultivars, independent of the N-level. The lower leaf appearance rate of the tetraploid cultivars coincided with a higher final leaf size (Table 1). Leaf size of tetraploids was on the average 24% greater than that of diploids. In the seedling stage, the effect of the lower leaf appearance rate was overcompensated by the bigger final leaf size. The lower leaf appearance rate however, restricted the number of axillary shoots of the tetraploid cultivars and thus the number of regrowing leaves after cutting. The impact on the total dry matter yield (shoots and roots) is depicted in Fig. 2. A small but significant ($P < 0.01$) higher yield (20% averaged over all treatments) of the tetraploid cultivars at harvest 1 turned into a lower yield at harvest 4 ($P < 0.05$). The differences at harvest 2 and 3 were not significant.

The yield difference between tetraploids and diploids at harvest 4 was small compared to the 40% lower tiller number of the tetraploid cultivars at that time. Apparently, the tetraploids compensated for a lower tiller number by a higher leaf elongation rate in the heterotrophic stage immediately after cutting or by a higher relative growth rate (RGR). RGR can be separated into three components: RGR = LWR · SLA · NAR.

LWR = leaf weight ratio (leaf/plant g/g)
SLA = specific leaf area (leaf area/leaf weight m^2/kg)
NAR = net assimilation rate (dry matter increment/leaf area $g/m^2/d$)

Leaf weight ratio

Because the stubble (2 cm height) remained after the cuttings, the LWR in this case stands for the ratio between the harvested shoots and the total of shoots and roots. The leaf weight ratio decreased

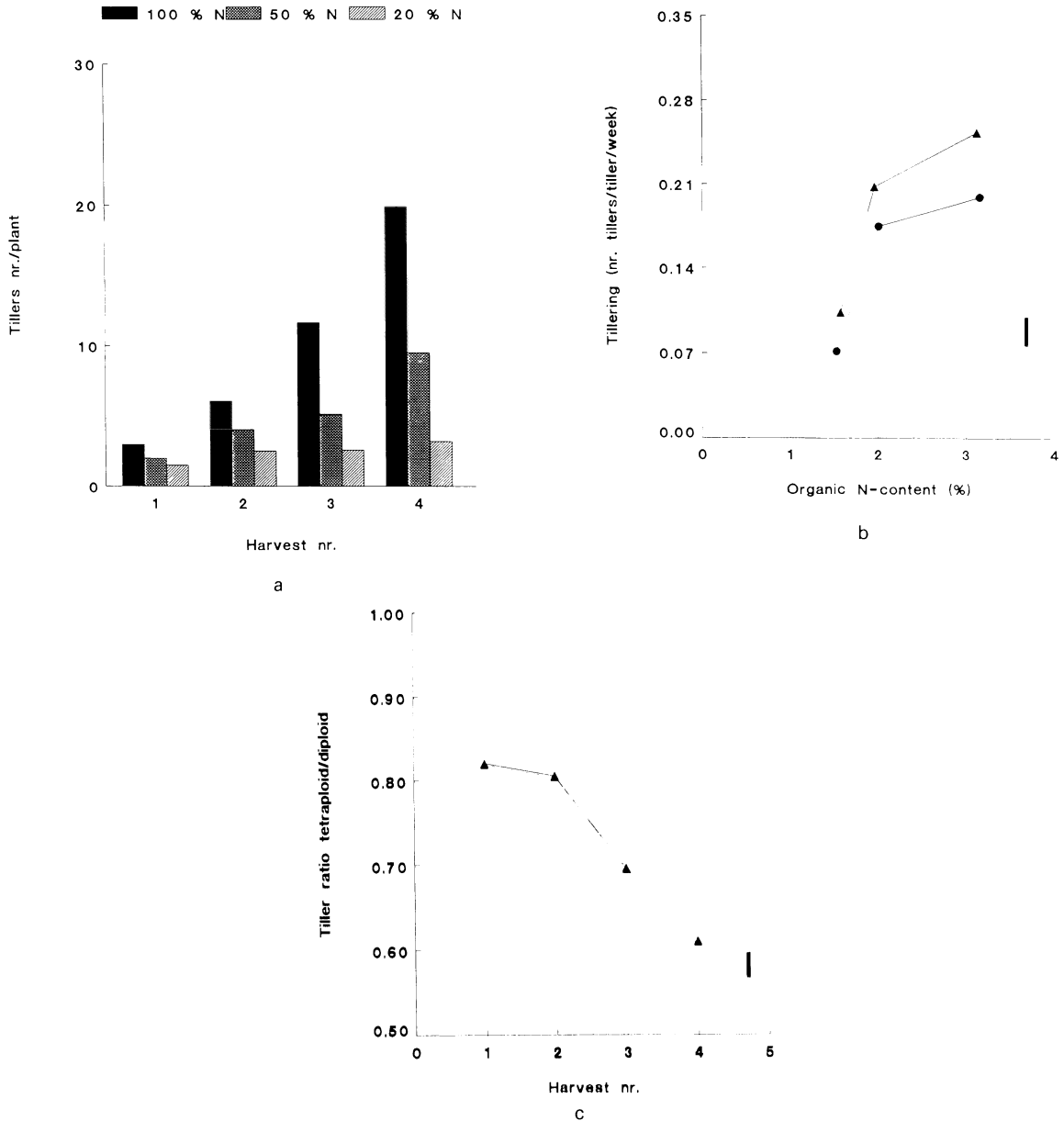

Fig. 1. **a**; Average cumulative tiller number of 12 diploid cultivars for 3 N-levels at 4 successive harvests. **b**; Average relative tillering rates for 12 diploid cultivars (*triangles*) and 12 tetraploid cultivars (*circles*) as a function of the organic N-content of the shoots. **c**; Ratio between the number of tillers in the tetraploid cultivars and the diploid cultivars averaged for the different N-applications. The *bars* present values for the SED.

almost linearly with the organic N-content (Nc in %) of the leaves (Fig. 3). The relations for the diploid and tetraploid cultivars were:

LWR = 0.172 + 0.176 Nc (r = 0.93) diploids
LWR = 0.204 + 0.162 Nc (r = 0.91) tetraploids

A decrease of the organic N-content directed more assimilates to the roots. There were no significant differences between ploidy levels. This excludes a high LWR of the tetraploid cultivars as a compensation factor for the low tillering rate.

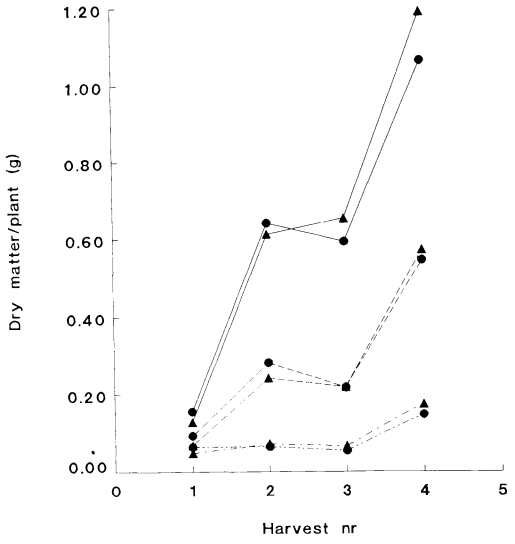

Fig. 2. Cumulated dry matter of shoots and roots at successive harvests for 12 diploid cultivars (triangles) and 12 tetraploid cultivars (*circles*) at 3 different N-application rates. Differences between ploidy levels were significant for harvest 1 ($P < 0.01$) and harvest 4 ($P < 0.05$).

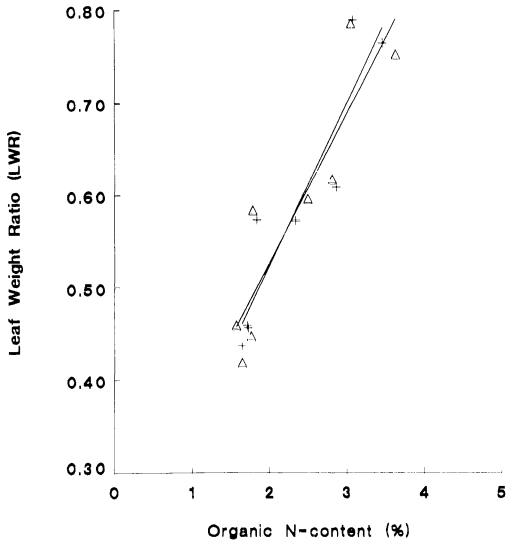

Fig. 3. Leaf weight ratios averaged over the 12 diploid cultivars (*crosses*) and 12 tetraploid cultivars (*triangles*) at different N-application rates and harvest dates, plotted against the organic N-content of the shoots.

Specific leaf area and dry matter content

Characterization of the specific leaf area is complicated by its dependence on leaf senescence, internal shading and temperature. In addition the leaves tend to curl once they have been cut, which restricts the use of optical devices for measuring leaf area. The measurement of leaf width and length seems the best out of the worst but is very time consuming because of the necessity of many replicates. The relations for the tetraploid and the diploid cultivars (Fig. 4) reveals:

SLA = 35.6 + 4.82 Nc (r = 0.79) diploid

SLA = 33.1 + 4.47 Nc (r = 0.78) tetraploid

The SLA of the diploid cultivars decreased from 49 at 3% organic nitrogen to $43\,m^2\,kg^{-1}$ at 1.5% nitrogen and for the tetraploid cultivars from 44 to $37\,m^2\,kg^{-1}$. The lower SLA of the tetraploid cultivars will have had an adverse effect on the relative growth rate. Therefore it is concluded that the observed yield compensation of the tetraploid cultivars is caused either by a high NAR or a high leaf elongation rate immediately after cutting, giving a relative high initial leaf area at the start of the exponential stage:

$$L = L_0 \exp(RGR_L \cdot t) \qquad (3)$$

L = leaf area (m^2).

Table 1. Average size (cm^2/leaf) of fully developed leaves of 12 tetraploid cultivars. Leaf size of the 12 diploid cv's is given as a percentage of that of the tetraploid leaf size at successive harvests for the 100%, 50% and 20% N-treatments. The SED was 0.25, 0.34, 0.48 and 0.60 for the successive harvests, respectively.

	Tetraploids (cm^2/leaf)				Diploids (% of tetraploids)			
Harvest no	1	2	3	4	1	2	3	4
Treatment								
100% N	6.07	12.05	12.27	19.44	68%	74%	77%	77%
50% N	5.28	6.22	7.09	10.15	76%	73%	81%	74%
20% N	3.72	3.28	3.98	6.63	77%	77%	91%	73%

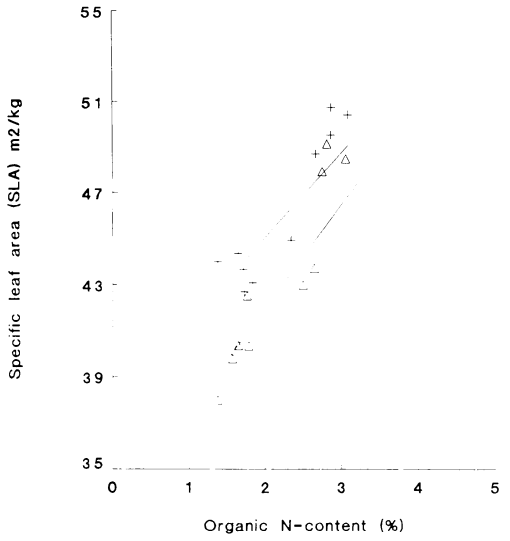

Fig. 4. Specific leaf areas averaged over 12 diploid cultivars (*crosses*) and 12 tetraploid cultivars (*triangles*) at different N-application rates and harvest dates, plotted against the organic N-content of the shoots.

L_0 is the initial leaf area at the start of the exponential stage. It is dependent on the number of tillers and the initial leaf elongation rate.
RGR_L is the relative increase of leaf area.

Five days after cutting, leaves of tetraploids were 8% longer than leaves of diploids which, considering a difference in leaf width of 17%, gave the tetraploid 26% more leaf area at the start of the exponential stage. The water content of the leaves may be considered as a direct repercussion of the cell wall elasticity and its potential to elongate. Fig. 5 shows the relations between the dry matter contents and the reciprocals of the organic N-content in the leaves for diploid and tetraploid cultivars, respectively.

Dc = 0.074 + 0.183 1/Nc (r = 0.93) diploids
Dc = 0.065 + 0.157 1/Nc (r = 0.94) tetraploids

The water content was about 16% higher in tetraploid cultivars than in diploid cultivars. The relative difference is slightly smaller at low organic N-content of the leaves.

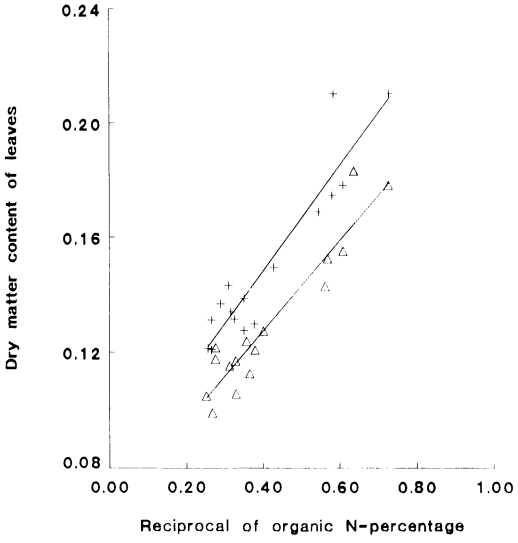

Fig. 5. Dry matter content averaged over 12 diploid cultivars (*crosses*) and 12 tetraploid cultivars (*triangles*) at different N-application rates and harvest dates, plotted against the reciprocal or the organic N-content of the shoots.

Light-saturated photosynthesis

For 6 randomly selected diploid and tetraploid cultivars it appeared that, at the high N-level, the light-saturated photosynthesis was about 30% higher for the tetraploid cultivars than for the diploid cultivars ($P < 0.05$). The low SLA (thick leaves), the identical N-content of the leaves on a dry matter basis and the high water content will have contributed to the higher light-saturated photosynthetic rate of the tetraploids cultivars compared to the diploid cultivars. A more detailed analyses of the cultivars Wendy (diploid) and Condesa (tetraploid) is depicted in Fig. 6. Whereas tetraploid cultivars in general have a higher light-saturated photosynthesis (Amax), these particular cultivars responded alike:

Amax = 0.336 + 0.224 Nc (r = 0.96) Wendy
Amax = 0.322 + 0.219 Nc (r = 0.92) Condesa

Condesa had a lower organic nitrogen content than Wendy, but this was compensated by a lower SLA, giving about the same organic nitrogen concentration on a leaf area basis. In this respect, Condesa was an exception for the tetraploids. Averaged over all cultivars, organic nitrogen contents were exactly equal for the diploid and the tetraploid cultivars. The difference in Amax between ploidy levels was therefore due to differences in SLA and water content. The advantage of a higher light-saturated photosynthesis (Amax) is relatively small, especially at low radiation flux densities or when full light

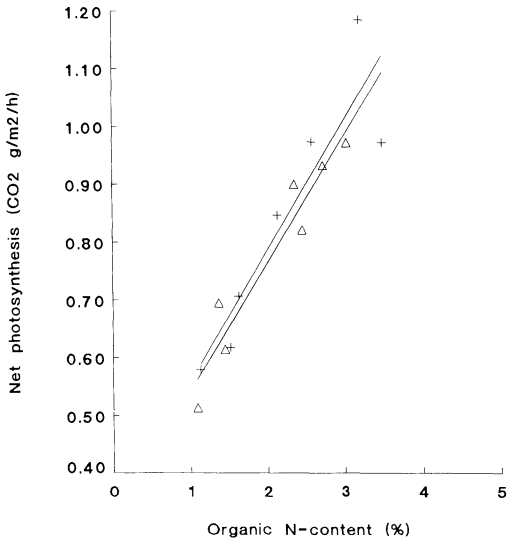

Fig. 6. Light-saturated photosynthetic rates of cv. Wendy (*crosses*) and cv. Condesa (*triangles*) plotted against the organic N-content of the leaves.

interception is reached at a LAI above 3. Though we did not quantify the impact on the RGR it seems likely that the 30% higher Amax of the tetraploids is fully counterbalanced by the 10–15% lower SLA.

Yield difference within ploidy levels

The interaction between the nitrogen addition rates and the genotypical yield differences is summarized in Figure 7. The regression of the cumulated fresh weight in 7 cuttings for the 50% (Fig. 7a) and 20% treatment (Fig. 7b) on the cumulated fresh weight at an optimal nitrogen addition rate (100%) show good correlations and similar ranking orders for the different genotypes. This indicates that a genotype with a relatively high yield at optimal nitrogen supply generally will give a high yield under sub-optimal nitrogen supply as well.

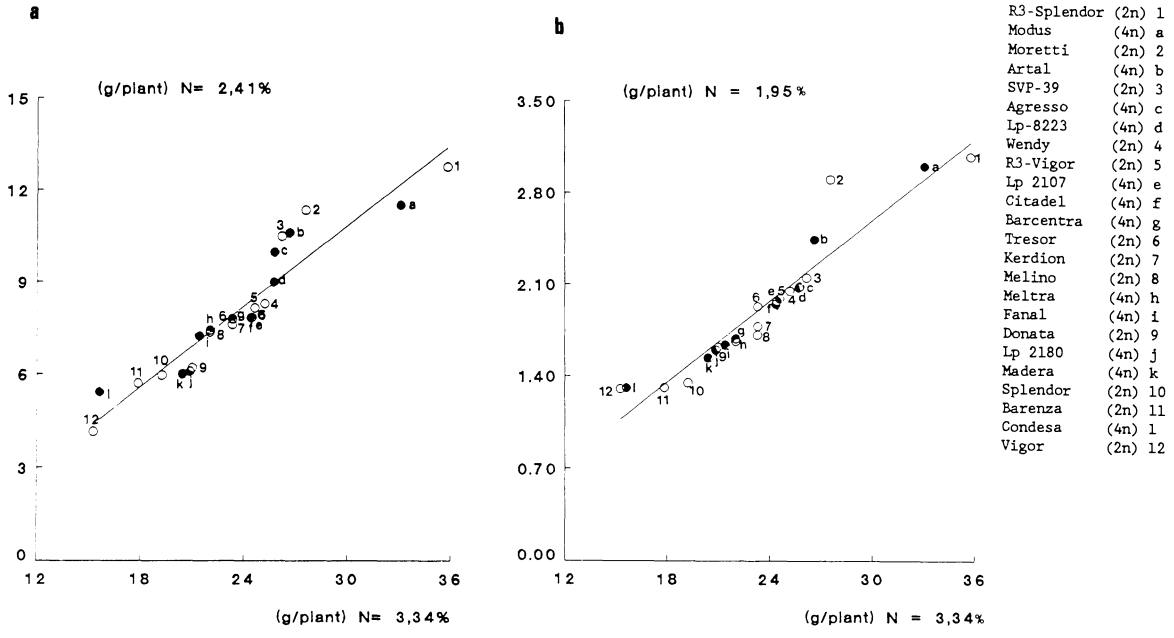

Fig. 7. Shoot fresh weight of different populations cumulated over 7 harvests at sub-optimal relative addition rates of nitrogen, plotted against the cumulative production at an optimal relative addition rate of nitrogen. The relative addition rates were based on a calculated supply ratio of 80% (**a**) and 50% (**b**).

Conclusions

The effect of the organic N-content on the RGR was about the same for both diploid and tetraploid cultivars. An increase of the organic N-content from 1% to 4% enhanced the leaf weight ratio by 100%, specific leaf area by 20% and light-saturated photosynthesis (of two cultivars only) by 100%. The positive effect of the latter is of minor importance because it is only slightly reflected in the net assimilation rate (Davidson *et al.*, 1986; Robson and Parsons, 1978). The increase of the SLA and the LWR, however are proportionally reflected in the RGR, which makes the increase of the LWR the most important factor in the response of growth to the N-supply.

With optimal N-application, the light-saturated photosynthesis of tetraploid cultivars was 30% higher than that of diploid cultivars.

The LWR of diploid and tetraploid cultivars was equal at the respective organic N-contents.

The SLA of tetraploids was 10–15% lower than that of the diploids and the final leaf size was 24% bigger. The bigger leaf size resulted in a lower leaf appearance rate and consequently a lower tillering rate. The absolute difference between the tiller density of diploids and tetraploids increased continuously. Still the yield of tetraploids was relatively high due to a higher heterotrophic leaf elongation rate immediately after cutting. The RGR in the subsequent exponential stage was similar to that of the diploids. It is to be expected that the low tillering rate will lead to a further decline in the yield of tetraploid cultivars at later harvests.

The high significance of morphological attributes with respect to yield potential provides good opportunities for breeding options because these attributes are easily quantified and are subject to only minor interactions between genotype and environment. This might explain why the ranking order for the yield of different genotypes remained the same at different nitrogen addition rates.

Caution should be taken to extrapolate these results to field conditions, primarily because root functioning is of minor importance on a nutrient solution and secondly because the presented results refer to experiments with mixtures rather than monocultures. Competition effects certainly contributed to the oberved results and as such the experiments should be considered as an evaluation of differences in persistency rather than a study to quantify potential production capacities of genotypes in monocultures.

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PLSO PN 169

Actual cytokinin concentrations in plant tissue as an indicator for salt resistance in cereals

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Key words: barley, benzyladenine, cytokinins, NaCl, regulation, salt resistance

Abstract

Growth rates of roots and shoots of hydroponically grown plants of a salt resistant variety of barley decreased rapidly after an exposure to 65 $M\,m^{-3}$ sodium chloride. This growth rate reduction tended to be temporarily and it was accompanied by a lowered shoot to root ratio. At the same time growth reduction took place, the cytokinin concentrations in root and shoot tissue decreased rapidly too, as they were measured by an enzyme-linked immuno assay. The addition of $5 \cdot 10^{-9}\,M\,m^{-3}$ benzyladenine (BA) retarded the decreases of growth rates, shoot to root ratio and internal cytokinin concentrations.

Growth rates of roots and shoot of identically grown plants of salt sensitive varieties of barley maintained their growth rates and shoot to root ratio after an exposure to NaCl for at least ten days in combination with unchanged levels of endogenous cytokinins. After *ca* 14 days NaCl induced severe decreases in RGR values, but not in shoot to root ratio. Addition of benzyladenine inhibited growth to some extent and enhanced the cytokinin concentration.

It was concluded, that cytokinin concentrations measured directly after exposure to NaCl form a promising screening method for salt resistance. The addition of BA made salt resistance varieties behave as salt sensitive varieties.

Abbreviations. BA, benzyladenine; i^6 Ade, isopentenyladenine; i^6 Ado, isopentenyladenosine; RGR, relative growth rate: S/R, shoot to root ratio; Z, zeatin; ZR, zeatin riboside.

Introduction

In previous papers we presented results verifying a growth model in which endogenous cytokinin levels play an important role. Plants of *Plantago major* L. ssp. *pleiosperma* (Pilger) reduced their relative growth rate (RGR) and shoot to root ratio (S/R) very rapidly after an exposure to a 50 times diluted nutrient solution (Kuiper, 1983; 1988). The quick growth responses correlated positively with the endogenous cytokinin concentrations of root and shoot tissue (Kuiper and Kuiper, 1988). The addition of $10^{-8}\,M\,m^{-3}$ benzyladenine (BA), a synthetic cytokinin, to the diluted nutrient solution, postponed the reductions in RGR, S/R and cytokinin concentrations for about ten days (Kuiper and Staal, 1987).

From our data we hypothesized, that a rapid growth reaction to a growth hampering factor in the rizosphere, may be mediated by a lowered cytokinin production (Kuiper *et al.*, 1988; 1989).

In order to elaborate this hypothesis further we report here upon the effects of NaCl on growth and cytokinin concentrations of several varieties of barley and wheat. The observation that cytokinin levels in plant tissue are sensitive to salt has already been published by Goodwin (1978) and Torrey (1976).

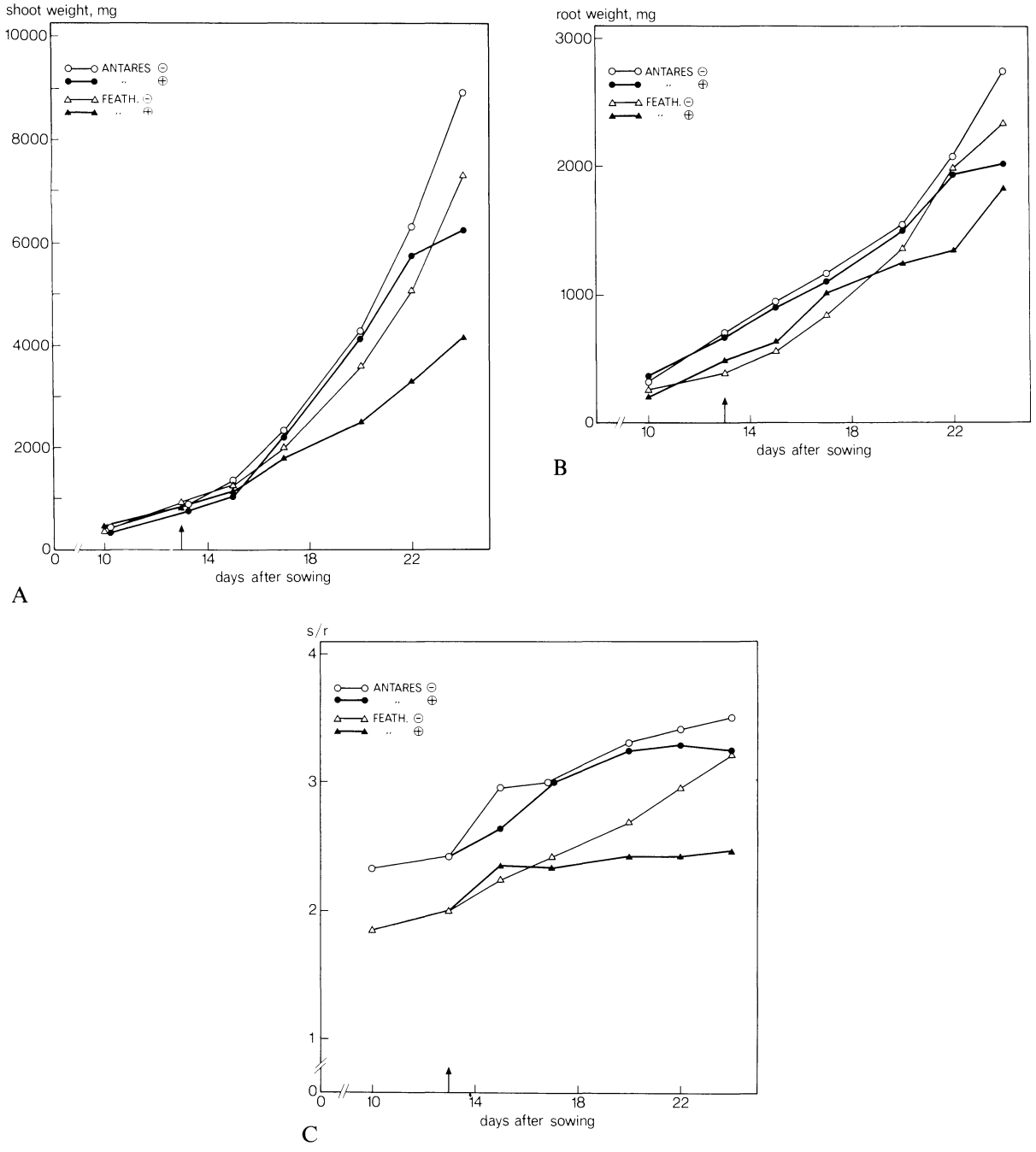

Fig. 1. Shoot growth (**A**), root growth (**B**) and S/R (**C**) of two varieties of barley with (+) and without (−) NaCl in nutrient solution.

Materials and methods

Growth conditions

Seeds of several salt resistant and salt sensitive varieties of barley and wheat (kindly supplied by ICARDA, the International Centre for Agricultural Research in Dry Areas) were germinated in moistened vermiculite and transferred to a vessel containing 30 L 25% strength nutrient solution ac-

cording Smakman and Hofstra (1982). Twenty plants were grown on each vessel and the nutrient solution, pH 6.0, was renewed twice a week. At day 13 after sowing half of the plants were supplied with 65 $M\,m^{-3}$ NaCl ± BA. The realized BA concentrations were 10^{-6}, 10^{-7}, $5\cdot10^{-8}$, 10^{-8}, $5\cdot10^{-9}$ and $10^{-9}\,M\,m^{-3}$. Growth conditions were as described by Kuiper and Staal (1987).

Growth

Ten plants were randomly chosen from each treatment. The volumes of the root systems and the total weights of the plants were measured three times a week. With aid of previously determined linear correlations between root volume and root fresh weight we were able to follow the growth of the shoot and the roots of the individual plants. From these data we calculated S/R and the RGR values of roots and shoots. All data were submitted to an analysis of variance to get proper indices of significance.

Cytokinins

The extraction, purification and determination by enzyme immuno assay method (EIA) were performed as described by Kuiper *et al.* (1989) and Vonk *et al.* (1986).

Results

Growth

Figure 1A–C forms a representative picture of the growth responses of a salt sensitive (*i.e.* Antares) and a salt resistant (*i.e.* Featherstone) variety of barley and wheat. After the addition of NaCl the reduction of the RGR appeared faster in the salt resistant lines than in the salt sensitive ones, but the responses in the latter ones were bigger and more permanent. In some of the studied salt resistant lines the RGR of the shoot showed a recovery at the end of the experiment (Table 1). The response of the S/R to NaCl is very characteristic for

Table 1. Relative growth rate (RGR) in mg g^{-1} fresh weight·day of shoot and roots of salt-tolerant and -sensitive barley and wheat varieties as affected by salinity. Salt was added on day 13 after sowing. RGR was determined for 2 conductive periods of 5 days after salt addition

Crop and variety	Tolerance	NaCl (65 mM)	RGR (root)		RGR (shoot)	
			Days after addition of salt			
			0–5	5–10	0–5	5–10
Barley						
Featherstone	+	−	155	163	192	210
		+	126[a]	121[a]	163[a]	172[a]
Calif. Mariout	+	−	168	171	170	181
		+	142[a]	140[a]	153[a]	150[a]
White Gatami	+	−	200	181	204	189
		+	213	215[a]	148[a]	174[a]
Club Mariout	+	−	175	184	183	195
		+	149[a]	168[a]	141[a]	171[a]
Antares	−	−	180	185	228	233
		+	171	95[a]	215	141[a]
Arivat	−	−	175	186	202	210
		+	179	111[a]	206	133[a]
Wheat						
Kharchia	+	−	196	172	209	181
		+	212[a]	168	164[a]	160[a]
Candeal	+	−	210	198	180	184
		+	215	189	148[a]	159[a]
Anza	−	−	187	162	205	191
		+	188	131[a]	191	135[a]

[a] Significant at the $P = 0.05$ level for differences between salt treatment and control.

Table 2. Time course of shoot to root ratio of salt-tolerant and salt-sensitive varieties of barley and wheat as affected by salinity. Salt was added at day 13 after sowing

Crop and variety	Tolerance	NaCl (65 m*M*)	Days after NaCl addition			
			0	4	9	11
Barley						
Featherstone	+	−	1.99	2.26	2.95	3.21
		+		2.36	2.41[a]	2.45[a]
Calif. Mariout	+	−	1.85	2.11	2.65	2.86
		+		2.03	2.15[a]	2.17[a]
White Gatami	+	−	1.45	1.31	1.34	1.66
		+		1.43	0.94[a]	0.96[a]
Club Mariout	+	−	1.67	1.58	1.68	1.82
		+		1.53	1.29[a]	1.18[a]
Antares	−	−	2.42	2.65	3.40	3.48
		+		2.96	3.28	3.23
Arivat	−	−	2.19	2.28	2.92	3.08
		+		2.35	2.80	2.86
Wheat						
Kharchia	+	−	1.54	1.65	1.95	1.98
		+		1.52	1.36[a]	1.26[a]
Candeal	+	−	0.98	1.07	1.08	1.12
		+		0.98	0.44[a]	0.58[a]
Anza	−	−	1.84	1.95	1.91	2.01
		+		1.98	1.84	1.89

[a] Significant at the $P = 0.05$ level for differences between salt treatment and control.

both types of varieties; a significant decrease of S/R in the salt resistant varieties within 7 days after salt addition, whereas the S/R in salt sensitive lines was stabilized irrespective of the treatment (Table 2).

The effects of several BA concentrations in the nutrient solution (± NaCl) on the growth (Table 3) and S/R (Table 4) of Featherstone are shown. The proper concentration of BA ($5 \cdot 10^{-9} M\,m^{-3}$) retarded the normally observed growth responses, resulting in a more or less stable S/R in NaCl + BA plants of Featherstone. BA effected negatively the growth of Antares (data not shown).

Cytokinins

The concentrations of zeatin (Z) and zeatin riboside (ZR) in root and shoot tissues of Featherstone (salt resistant) and Antares (salt sensitive) are presented in Table 5 and the data on

Table 3. Effect of addition of benzyladenine on day 13 on the relative growth rate (RGR on a fresh weight basis in mg/g·day) of salt-affected barley of the tolerant variety Featherstone. Salt was added on day 13 after sowing

Treatment		RGR (root)		RGR (shoot)	
NaCl (65 m*M*)	BA (*M*)	Days after addition of salt and/or BA			
		0–5	5–10	0–5	5–10
−	−	185[a]	183[a]	198[a]	210[a]
+	−	146	151	163	172
+	10^{-9}	171[a]	159	183[a]	180
+	10^{-8}	189[a]	123[a]	201[a]	158
+	10^{-7}	151	121[a]	128	100[a]
+	10^{-6}	123	86[a]	102	74[a]

[a] Significant at the $P = 0.05$ level for differences between controls (+ or − salt) and BA addition (control, + salt).
RGR was determined for 2 consecutive periods of 5 days after salt addition.

Table 4. Effect of addition of benzyladenine (BA) on day 13 on the shoot/root ratio of salt-affected barley of the tolerant variety Featherstone. Salt was added on day 13 after sowing

Treatment		Days after addition of salt and/or BA			
NaCl (65 m*M*)	BA (*M*)	0	4	9	11
−	−	2.01	1.96[a]	2.35[a]	2.54[a]
+	−		1.66	1.73	1.71
+	10^{-9}		1.73	2.11[a]	2.06[a]
+	10^{-8}		2.05[a]	2.10[a]	1.98[a]
+	10^{-7}		1.15	1.42[a]	1.19[a]
+	10^{-6}		1.45[a]	1.18[a]	1.02[a]

[a] Significant difference between controls (+ or − NaCl) and BA addition (control, + NaCl) at the $P = 0.05$ level.

Table 5. Concentrations of zeatin and zeatin riboside (pmol·g^{-1} FW) in shoot and root tissue of plants of a salt resistant (Featherstone) and a salt sensitive (Antares) variety of barley. T, days after transfer to a nutrient solution with 65 *M* m^{-3} NaCl and/or 5·10^{-9} *M* m^{-3} BA, $n = 10$; nd, not determined

Treatment		T	Shoot		Root	
			Z	ZR	Z	ZR
Featherstone						
Control		0	38 ± 2.1	25 ± 3.1	40 ± 3.4	21 ± 1.1
		1	41 ± 3.2	23 ± 1.9	42 ± 3.1	25 ± 2.1
		2	44 ± 3.1	27 ± 2.3	45 ± 4.1	29 ± 3.0
		4	48 ± 3.2	32 ± 1.8	49 ± 4.2	32 ± 2.6
+ BA		1	51 ± 4.3	30 ± 2.1	56 ± 4.2	32 ± 2.8
		2	nd	nd	nd	nd
		4	65 ± 4.8	41 ± 2.6	61 ± 5.2	39 ± 3.2
	+ NaCl	1	25 ± 1.6	14 ± 0.6	24 ± 1.8	14 ± 1.1
		2	18 ± 1.4	12 ± 0.6	21 ± 1.6	14 ± 1.1
		4	23 ± 2.5	17 ± 1.6	27 ± 2.1	17 ± 1.7
+ Ba	+ NaCl	1	38 ± 2.1	27 ± 2.0	39 ± 2.6	28 ± 3.0
		2	47 ± 4.0	31 ± 2.2	51 ± 3.8	39 ± 2.9
		4	51 ± 4.6	36 ± 2.3	54 ± 3.8	37 ± 3.1
Antares						
Control		0	28 ± 1.4	13 ± 1.1	39 ± 2.3	13 ± 1.0
		1	29 ± 1.8	11 ± 0.6	37 ± 2.1	10 ± 0.4
		2	33 ± 2.6	16 ± 1.0	43 ± 2.9	13 ± 0.8
		4	33 ± 2.4	15 ± 0.8	41 ± 2.7	14 ± 1.1
+ BA		1	35 ± 2.8	17 ± 0.9	42 ± 3.4	15 ± 0.5
		2	39 ± 3.0	21 ± 1.2	47 ± 3.1	18 ± 0.8
		4	42 ± 2.9	24 ± 1.5	50 ± 3.8	21 ± 1.3
	+ NaCl		31 ± 2.8	13 ± 1.1	34 ± 2.4	12 ± 0.6
		2	29 ± 1.9	11 ± 0.7	36 ± 2.3	14 ± 0.7
		4	30 ± 2.1	15 ± 0.8	43 ± 2.7	17 ± 1.1
+ BA	+ NaCl	1	34 ± 3.0	16 ± 1.2	41 ± 3.1	15 ± 0.8
		2	36 ± 2.9	18 ± 1.1	46 ± 3.2	17 ± 0.8
		4	41 ± 3.2	24 ± 2.0	48 ± 3.1	22 ± 1.5

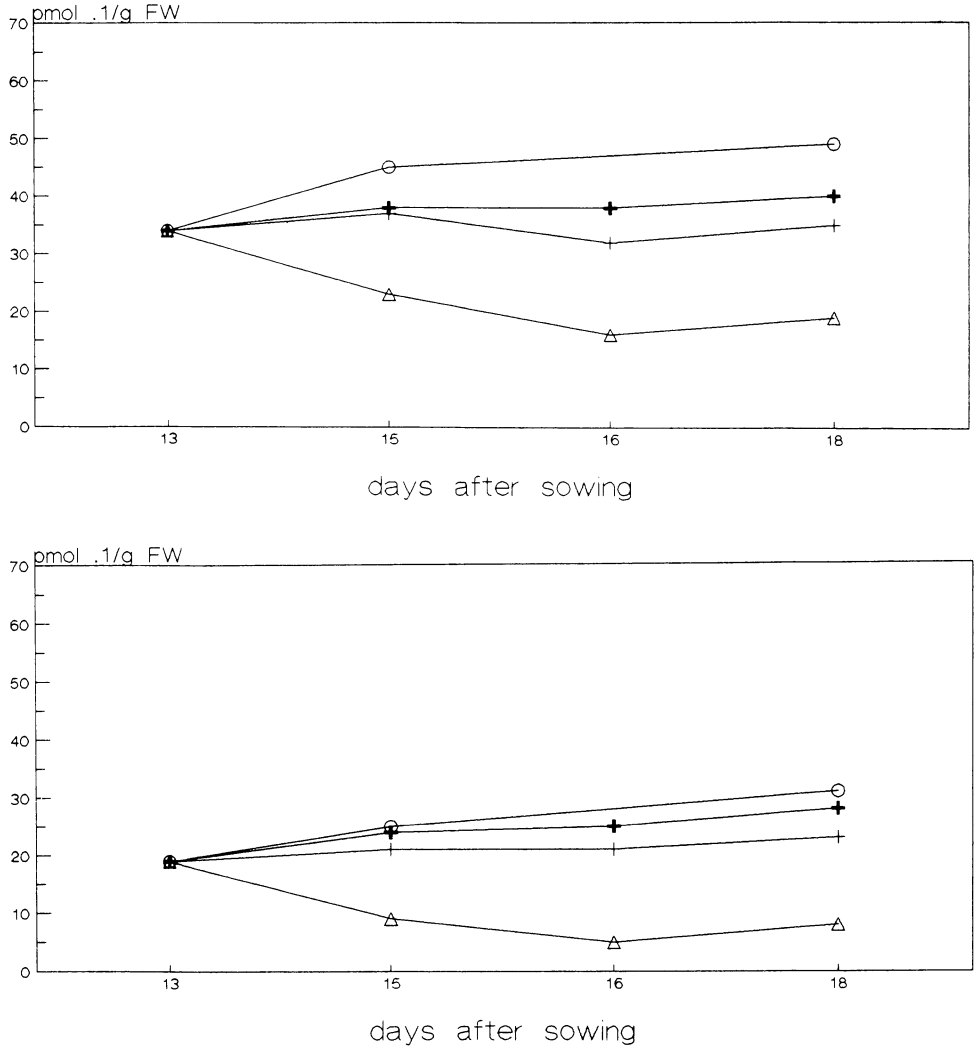

Fig. 2. Concentrations (pmol·g^{-1}FW) of isopentenyladenine (**A**) and isopentenyladenosine (**B**) in the shoot of Featherstone. +, control; △, NaCl; ○, BA; +, NaCl + BA.

isopentenyladenine (i^6Ade) and isopentenyladenosine (i^6Ado) from shoot material of Featherstone in Figure 2A–B. Concentrations of Z and ZR were significantly lower in salt resistant plants. A period of 48 h seems to be sufficient to establish new stable levels of Z and ZR, approximately half of those in the control plants. BA addition to the nutrient solution increased the Z and ZR concentrations in both root and shoot tissue of Featherstone. The effect of BA in NaCl treated plants was maintaining the levels of Z and ZR at the control level. On the contrary, Z and ZR concentrations in salt sensitive plants did not react to salt during the time of the experiment, but BA increased the levels of Z and ZR.

The responses of the i^6Ade and i^6Ado concentrations were quite comparable with Z and ZR results. As an illustration we only show the i^6Ade and i^6Ado concentrations of the Featherstone shoot.

Discussion

Four main conclusions can be drawn from the present results:

NaCl affected RGR and S/R of salt resistant

plants immediately after the beginning of the salt treatment in contrast to salt sensitive plants.

NaCl decreased the cytokinin concentrations in root and shoot tissue of salt resistant plants, but not of salt sensitive plants.

BA addition increased endogenous cytokinin concentrations.

BA addition could overcome temporarily NaCl induced effects in salt resistant plants.

In summary, salt resistant individuals decreased their RGR and S/R in combination with their internal cytokinin concentrations shortly after the addition of 65 $M\,m^{-3}$ NaCl in the nutrient solution. These effects are compatible with our definition of cytokinin mediated growth. So we assume that a decrease in cytokinin concentrations is the first response to salt, followed by a reduction of the RGR values. The proper concentration of BA in the nutrient + NaCl solution maintained the growth parameters by means of the internal cytokinin concentration (Kuiper and Staal, 1987) and prolonged experiments demonstrated that BA + NaCl treated salt resistant plants behaved like salt sensitive plants; a delayed but permanent reduction in RGR.

Barley is thought to use multiple mechanisms to cope with the problems of osmotic imbalance, sodium toxicity and ion imbalance, that are brought about when plants are grown in saline conditions. These mechanisms include extrusion of sodium across the plasma membrane of root cortical cells, storage of excess sodium in vacuoles, partial exclusion of Na ions from the leaves, redistribution of potassium from older to younger tissue and a tendency to concentrate sodium in older tissues (Greenway and Munns, 1980; Jeschke, 1984; Jeschke *et al.*, 1983; Lynch *et al.*, 1982; Pitman *et al.*, 1981). It is likely, that a number of specific molecular mechanisms are responsible for these mechanisms and that initially exposure to salt will activate or induce at least a part of these processes with an enhanced need for energy, which will limit growth during adaptation. The cytokinin production may form the build-in trigger for tuning growth and the salt resistance mechanisms.

The effects of NaCl on salt sensitive plants is not mediated by cytokinins, because a growth reduction appeared before any decrease in cytokinin levels was observed. The incapacity of BA to increase RGR values of salt treated individually also indicates a different role for the cytokinins in salt sensitive plants. Salt will penetrate into all parts of the plant and eventually hamper the growth.

Determination of the cytokinin concentration may form a useful tool in a screening procedure for salt resistance in cereals.

Acknowledgements

We are grateful to Dr H M Dekhuijzen, Mr C R Vonk and Mr E Davelaar, all from the Centre for Agrobiological Research (CABO) in Wageningen for their helpful advice and assistance in the Immuno-Assay work. These investigations were supported by the Foundation of Technical Sciences (STW), which is subsidized by the Netherlands Scientific Organization (NWO).

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PLSO PN 170

A procedure for quick screening of wheat cultivars for salt tolerance

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Key words: criteria, germination, maturity, salinity, shock, salt-affected field, seedling growth, soil culture, solution culture, type of salinity

Abstract

A quick and reliable procedure for mass screening of wheat genotypes for salt tolerance has been developed. Four-day old seedlings raised in gravel culture are transferred to foam plugged holes in polystyrene (thermopal) sheets suspended over 100 dm^3 of half strength Hoagland solution in plastic lined galvanized iron tanks (120 × 90 × 15 cm). Two days after transplanting, salinization of the solution is started with NaCl in 8 equal increments to 0, 200, 300, and/or 400 mol m^{-3} concentrations and therein plants are allowed to grow for four weeks. Solutions are aerated 8 hours day^{-1} and renewed weekly. Relative fresh shoot yield calculated as percentage of the control is the main selection criterion along with visual scoring at high salinity. In one set, up to 40 cultivars with 12 plants each, can be screened thrice during a growing season. Related studies were conducted to standardize the procedure. Fourteen wheat cultivars screened under the developed system gave highly reproducible results, closely comparable with those of grain yields in salinized soil beds and the saline field, which authenticated the validity of the technique. Salt tolerance rating of the cultivars based on different selection criteria was compared. Overall pattern of varietal behaviour remained fairly constant under different situations. Tolerance at germination stage, however, did not always reflect salt tolerance at seedling stage. Dilemma of change in salt tolerance pattern of some cultivars and problems pertinent to screening have been highlighted.

Introduction

Rehabilitation of the salt affected wastelands of the world can be accomplished either by adopting reclamation measures involving physical, chemical and hydrological approaches or through the biological approach involving development and introduction of salt tolerant plant species of economic importance and use of salt tolerant varieties of the cultivated crops. The latter approach has special relevance to countries like Pakistan where out of the 5.7 million hectares of the salt affected land, over one million hectares are actually under irrigated agriculture (Choudhri *et al.*, 1978) with wheat as the major crop. In this area, the average decrease in wheat yield varies from 37% on slightly salt affected to 69% on moderately salt-affected lands. An annual loss of Rs. 14,000/- million was estimated for the entire irrigated area of the country on account of decrease in production level due to salinity (Qayyum and Malik, 1985).

It is obvious that the major objective of screening for salt tolerance is to select genotypes that would give maximum economic yields on saline fields under the given climatic conditions (temperature, humidity, *etc.*), soil types (level and type of salinity, and associated problems of waterlogging, sodicity, permeability, bulk density, pH *etc.*), and management practices. There is a lack of understanding of the mechanisms of salt tolerance and how these are influenced by various environmental factors (see Nieman and Shannon, 1976; Levitt, 1980) and with ontogeny. Screening for salt tolerance in wheat has been attempted in solution culture salinized with NaCl alone, NaCl + $CaCl_2$ or dilutions of sea water; at germination, seedling or maturity stages;

in petri dishes, pots, water tanks or sand dunes (McGuire and Dvorak, 1981; Qureshi *et al.*, 1980; Shannon, 1978; Srivastava and Jana, 1984). Some workers have used artificially salinized soil in pots, containers or micro plots (Douglas and Deway, 1962; Qureshi *et al.*, 1980). Irrigation of fields with saline waters has also been tried (Epstein *et al.*, 1980) while there have been attempts to screen germplasm in naturally saline areas (Srivastava and Jana, 1984). Some of the shortcomings of these methods are discussed by Nieman and Shannon (1976) and Shannon (1984). In spite of the claims of success (Stavarek and Rains, 1984) techniques like cell culture do not have much promise as salt tolerance is a whole plant phenomenon. In cell, solution, sand and artificially salinized soil culture some of the problems associated with salinity such as nutrient imbalance, pH and poor aeration *etc.* are usually eliminated while, screening in the naturally saline fields suffers from the lack of control over environments and root zone heterogeneity both in time and space.

Based on our past experience we have tried to develop a rather simpler solution culture screening technique and the results obtained at different times have been compared with the controlled experiments in soil and with the field data.

Materials and methods

To find answers to a number of questions pertinent to the development of a reliable and efficient method of mass screening of wheat cultivars the following experiments were conducted: (1) effect of type of salinity on wheat growth, (2) effect of stage of salinization on wheat growth, (3) screening at germination stage, (4) screening at seedling stage (4 sets), (5) screening at maturity in soil beds, and (6) screening under natural field conditions. The details of these experiments are as follows:

Effect of type of salinity

Effect of two concentrations (150 and 300 meq l^{-1}) of three types of salinity *i.e.* NaCl + - $CaCl_2$ (1:1; equivalent basis), and a salt mixture (Na_2SO_4:NaCl:$CaCl_2$:$MgCl_2$ @ 10:5:4:1) on the growth and yield of two wheat varieties *i.e.*, LU 26S (tolerant) and Pothohar (sensitive) was studied in gravel culture in plastic containers using $\frac{1}{2}$ Hoagland's solution (Qureshi *et al.*, 1977). Each container had 12 hills (6 for each cultivar) with three plants per hill. Grain and straw yields and growth components were noted (3 hills).

Effect of stage of salinization on wheat growth

Twenty kg of sandy loam non-saline soil was filled into 12 plastic tubs and partitioned into four equal parts (replications). Four plants of LU 26S variety were grown in each part. Salinity (150 mol m^{-3} NaCl) was added in a single dose each time at eleven different stages of growth while, one tub was kept as control. Calculated amounts of NaCl dissolved in sufficient amounts of water were used to irrigate the soil to field capacity at each stage.

Screening at germination stage

Thirteen wheat varieties were tested for germination at 0, 100, 200, 300, and 400 mol m^{-3} NaCl concentrations in $\frac{1}{2}$ Hoagland's solution using plastic trays (30 cm dia. 5 cm depth) containing foam sheets. Care was taken to maintain salt concentration constant throughout. The experiment was replicated twice and lasted for 20 days.

Screening at the seedling stage

Seedlings were raised in plastic coated iron trays (60 × 30 × 5 cm) filled with silica gravels (4 mm dia.). For the first 3 days distilled water was applied and trays remained covered. Later on, $\frac{1}{2}$ Hoagland solution No. 1 (Hoagland and Arnon, 1950) was applied until seedlings were transplanted at 2 leaf stage (3–4 days after germination). Two seedlings per hole were held in place with foam in thermopal sheets suspended over 100 dm^3 of $\frac{1}{2}$ Hoagland nutrient solution in galvanized painted plastic lined iron growth tanks (120 × 90 × 15 cm) supported on iron stands 90 cm above ground. The solution was aerated during the day time. After two days of seedling establishment, sodium chloride was added to salinize the medium in increments of 25 mol m^{-3}

Table 1. Effect of types and levels of salinity on growth and yield of wheat crop (average of two cultivars and three hills)

	Salinity types and levels						
	Control	NaCl	NaCl + $CaCl_2$ 1:1	Salt mixture	NaCl	NaCl + CaCl 1:1	Salt mixture[a]
		150 mol m^{-3}			300 mol m^{-3}		
Nol. of tillers $hill^{-1}$	8.33a	6.8ab	6.00bc	6.17bc	4.67d	5.83bcd	5.17cd
Height of shoot cm	87.50a	70.17c	77.00b	72.50bc	44.33e	56.33d	56.50d
Grain yield g $hill^{-1}$	6.48a	1.73c	5.65a	3.76b	0.16e	1.09cd	0.75d
Straw yield g $hill^{-1}$	11.21e	4.27c	7.73b	6.59b	2.18c	3.19c	3.74c

[a] = Na_2SO_4 + NaCl + $CaCl_2$ + $MgCl_2$ @ 10:5:4:1 equivalent basis.
Mean values followed by the same letters within a row do not differ significantly (P = 0.05).

Table 2. Response of wheat to salinity shock (150 mol m^{-3} NaCl) at various growth stages

Growth stages	Tillers $hill^{-1}$	Height of main shoot (cm)	No. of ears $hill^{-1}$	Grain yield $hill^{-1}$ (g)	Straw yield $hill^{-1}$ (g)
Control	21.33ab	76.67a	19.67a	14.06a	30.07a
After emergence	5.00d	37.33f	4.33d	0.93e	4.98e
One shoot	8.67d	45.33e	6.00cd	1.21e	7.17de
Tillering begins	17.67bc	50.00b	9.33bc	4.07d	12.67cd
Tillers formed	16.67bc	53.33cd	9.33bc	4.37d	10.27cde
Tillers formed	14.67c	50.33d	7.67cd	4.18d	9.46cde
1st node formed	19.67ab	55.33c	9.67bc	4.45d	12.38cd
2nd node formed	19.67ab	55.33c	7.67cd	3.84d	11.89cd
Stem elongated	18.33bc	54.33c	8.67bcd	2.54de	14.36c
Boot	22.67a	51.67cd	11.67b	1.07e	11.33cd
Ears emerged	23.33a	66.67b	17.33a	9.28b	22.00b
After earing	24.00a	68.67b	16.33a	6.62c	25.76ab

Mean values followed by the same letters do not differ significantly within a column (P = 0.05).

per 24 hours up to the final salinity levels (0, 100, 200, 300 or 400 mol m^{-3} NaCl). The solution was checked frequently and changed when required. Each variety was replicated a number of times depending upon the number of varieties to be tested. The experiment was repeated in 4 sets (twice at Faisalabad in a net house without any environmental control, and twice at UCNW, Bangor, U.K. in a green house with temperature 15–25°C, 16 hours photoperiod of natural day light supplied by 400 w SON-T sodium vapour lamps). In Bangor, 0.1 mol m^{-3} $CaSO_4 2H_2O$ in deionized water was used for raising seedlings in growth cabinet and a modified 'Phostrogen' solution was used in 25 dm^3 grey plastic containers for seedling growth. The seedlings were harvested after 3–4 weeks of growth for measuring fresh and dry weights and other growth parameters.

Studies at maturity in salinized soil beds

A sandy loam soil salinized with NaCl to ECe 10 and 20 dSm^{-1} was filled in elevated concrete beds (450 × 90 × 60 cm, above ground) to a depth of 30 cm. Soil was watered and allowed to settle down and then fertilized with 50 kg P and 100 kg N ha^{-1} (2 splits). Seed was directly sown in soil in 90 cm

Table 3. Effect of NaCl salinity on the germination percentage of selected wheat cultivars (average of two replicates, fifty seeds)

Cultivars	Salinity levels (mol m^{-3} NaCl)				
	0	100	200	300	400
	Germination percentage				
Blue Silver	94	100	82	33	7
Punjab-81	98	100	73	34	10
Pak-81	94	97	76	23	10
Bahp-79	94	87	78	34	17
Lyp-73	90	106	80	26	9
Pothohar	98	83	61	18	2
LU 26S	100	100	84	37	17
SA-75	98	100	77	22	3
Indus-79	90	102	80	24	2
Pavan	96	89	70	20	6
Chenab-79	96	91	77	20	6
PARI-73	96	100	73	17	6
Sandal	92	104	79	15	2
Arz	—	—	—	—	—
LU 26	—	—	—	—	—
Mean	95	97	76	25	8

long rows and thinned to 6 plants per row. Number of replications was three. Irrigation was done with canal water till maturity.

Screening under field conditions

The soil in the field was silt loam, having $CaCO_3$ 3%, organic matter 0.8%, CEC 12.8 meq 100 g^{-1}, ECe 4–24 dSm^{-1}, pH 7.9–8.7, SAR 7.1. The soil was divided into 24 plots (replications) measuring 10 × 9 m.

The area had salinity variations of ECe < 4, 4–8, 8–12 and 12–24 dSm^{-1} in 4.2, 54.1, 37.5 and 4.2 percent of the plots, respectively. Thirteen cultivars were hand drilled after seed bed preparation, each in 4 rows, 9 m long with row to row distance of 30 cm. The crop was irrigated with canal water (EC 0.4 dSm^{-1}). The middle part of the two central rows (5 m length) was harvested for yield data.

Results and discussion

Effect of type of salinity

As expected, NaCl proved more toxic than NaCl + $CaCl_2$ and the salt mixture as was reflected in the significantly lower mean shoot height, grain yield and straw yield with NaCl compared with other salt treatments at both the salinities except for the straw yield at 300 mol m^{-3} NaCl salinity (Table 1). Although NaCl + $CaCl_2$ treatment showed better yield/growth than the salt mixture, the difference was significant only in the case of grain yield at lower salinity. The salt mixture used was close to the natural soil conditions in Pakistan but the use of Ca^{2+} and SO_4^{2-} at high concentrations causes precipitation of $CaSO_4 2H_2O$ leading to a decrease in salinity. Use of $CaCl_2$ along with NaCl has been preferred by a number of workers for physiological studies and for screening work (see Maas and Hoffman, 1977; Imamul Haq and Larher, 1983; Gorham *et al.*, 1984). This also matches the field soil conditions more closely. However, for practical reasons, the use of the single salt NaCl is preferred for situations like those in Pakistan where more than 60% of the salt affected soils (80% in the Punjab) are sodic as well.

Response of wheat to salinity shock at various growth stages

Data presented in Table 2 clearly indicate that the two early stages (just after emergence and 'one shoot') and the 'boot' stage were the most sensitive ones as the grain yields were significantly decreased when salinity was applied at these stages. Other growth parameters, *i.e.* number of tillers per hill, height of the main shoot, number of ears per hill and straw yield were also the lowest in the case of early salinization but not in the case of salinity application at boot stage. Thus, to reduce the time required for screening, salinization at early stage can be considered feasible.

Screening at the germination stage

Germination progressively decreased above 100 mol m^{-3} NaCl concentration and it fell to below adequate level (25%) at 300 mol m^{-3} as against 76% at 200 mol m^{-3} NaCl concentration (Table 3). Intervarietal differences were observed in tolerance at germination and, at 300 mol m^{-3} NaCl, it ranged from 15 to 37% of the respective controls of various varieties. Although Fryxell (1954)

advocated screening at germination as a useful technique, Abel and Mackenzie (1964) did not find relationship between tolerance at germination and later growth stages (also see Table 5).

Screening at the seedling stage

Many research workers (Epstein *et al.*, 1980; Mckimmie and Dobrenz, 1987; Ponnamperuma, 1984; Qureshi *et al.*, 1982; Shannon, 1978) have tried to develop rapid screening methods for salt tolerance at the early growth stages of different crops.

The major questions which remain to be answered in this respect are: (1) the suitability of various growth criteria, (2) reproducibility of the results using a particular technique, and (3) the validity of the technique in terms of field applications. These questions are taken up here in the context of the technique being used by us.

Criterion for selection

In the case of grain crops such as wheat, absolute grain yields of different varieties under a given set of soil salinity and climatic conditions form the basis of their comparative salt tolerance. At seedling stage, however, use of absolute fresh/dry shoot or root weights as the selection criteria may not be feasible because of the inherited differences in the absolute biomass production capacity of various varieties. Visual appraisal of salt injury to various cultivars is sometimes misleading, particularly in the case of cultivars which may partition salts into older leaves only (Rashid, 1986). Also, the visual observation largely depends upon the art of the worker and is rather vague.

The relative shoot dry weight yield (per cent yield of the respective controls) at high salinity is tentatively taken as the standard (most rational) criterion for determining relative salt tolerance of wheat cultivars, and the results based on other criteria (growth components) are evaluated against it (Table 4). The parameters like absolute plant height, number of tillers and green leaves were not significantly correlated with the respective shoot yields but the relative shoot yield was significantly positively correlated with the absolute shoot yield and relative root yields. However, root development in the hydroponic system is not comparable with the soil system and sometimes there was only a little or no effect of salinity on root mass in some cultivars. Therefore, root yields cannot be used as the selection criterion while both the absolute and relative yields could probably be used with equal confidence in the case of wheat.

However, the frequency distribution pattern of various varieties were classified in salt tolerance groups on the basis of relative growth rates.

Visual scoring and plant mortality at low salinity showed inconsistent results with respect to salt tolerance but, at high salinity of 400 mol m^{-3},

Table 4. Relationship among various growth parameters of wheat plants grown in saline media (200 m*M* NaCl)

Variables			Correlation coefficient
Absolute shoot yield	*vs*	Absolute root yield	0.775**
Absolute shoot yield	*vs*	Absolute plant height	0.499
Absolute shoot yield	*vs*	Absolute tillers	−0.305
Absolute shoot yield	*vs*	Absolute green leaves	0.246
Absolute shoot yield	*vs*	Relative root yield	0.775**
Relative shoot yield	*vs*	Relative plant height	0.417
Relative shoot yield	*vs*	Relative tillers	−0.138
Relative shoot yield	*vs*	Relative green leaves	0.214
Relative shoot yield	*vs*	Relative plant mortality	0.237
Absolute shoot yield	*vs*	Relative shoot yield	0.864**
Absolute root yield	*vs*	Relative root yield	0.935**
Absolute plant height	*vs*	Relative plant height	0.885**
Absolute green leaves	*vs*	Relative green leaves	0.573*

* = Significant ($P = 0.05$)
** = Highly significant ($P = 0.01$)

Table 5. Rating of wheat cultivars for salt tolerance at different stages of growth

Cultivars	Germination stage		Seedling stage						Maturity stage soil beds		Field trials	Final grading
			Set-I		Set-II		Set-III		Set-I			
	L	H	L	H	L	H	L	H	L	H		
Blue Silver	T	T	T	T	T	T	T	T	MT	T	MT	T
Pb-81	MT	T	MS	S	MT	S	MS	MS	S	MT	MT	S
PARI-73	MT	S	T	MT	T	MT	MS	MS	T	MS	S	MT
Pak-81	T	MS	MT	MT	MT	S	T	MT	MS	MS	MS	MT
Chenab-79	T	MS	MS	MS	MS	S	MS	MT	MT	MT	T	MT
Bahp-79	T	T	MS	MS	S	MS	T	T	S	S	T	MS
Lyp-73	T	MT	MT	T	MS	MT	MT	MT	T	MT	T	T
Pothohar	MT	S	MS	S	S	S	S	S	MT	S	S	S
LU 26S	T	T	T	T	T	MT	MT	MT	T	T	T	T
Pavan	MT	MS	S	MS	MT	MS	S	S	MT	S	MS	MS
SA-75	T	MS	MT	MT	MT	T	MS	MS	MS	S	MS	MT
Arz	—	—	MT	MT	MS	S	MT	MT	S	MS	—	MT
Sandal	T	S	S	S	—	—	—	—	S	MS	S	S
LU 26	T	—	S	MS	—	—	—	—	MS	MT	—	MS
Indus-79	T	MT	—	—	S	MS	S	S	—	—	MT	MS
LU60-1	T	—	—	—	MS	MT	MT	MT	—	—	—	MT

Salinity levels: L (low), H (high)
Tolerance class: S (sensitive); MS (moderately sensitive); MT (moderately tolerant); T (tolerant).

Table 6. Comparative performance of wheat cultivars during screening experiments

	Set-I			Set-II NaCl				Set-III		Soil beds ECe dSm^{-1}		Field studies Kg ha^{-1}
	200 R.S.	400 Visual scoring	400 Mortality (%)	200 R.S.	300 R.S.	400 Visual scoring	400 Mortality (%)	150 R.S.	300 R.S.	10 Grain g 6	20 Yield P^{-1}	
Blue Silver	66	15	(50)	59	28	24	(40)	75	35	15.8	9.8	1591
Pb-81	34	0	(100)	48	18	18	(60)	36	16	8.3	6.1	1644
PARI-73	55	10	(67)	66	21	22	(50)	39	18	20.1	5.4	1418
PAK-81	49	9	(67)	48	17	26	(40)	46	23	15.0	5.7	1494
Chenab-79	34	8	(67)	46	15	6	(80)	33	20	17.7	6.3	1906
Arz	52	12	(67)	41	15	10	(80)	41	19	11.8	5.8	—
Bahawalpur-79	35	2	(83)	34	20	4	(90)	49	25	11.9	5.3	1807
Lyp-73	48	18	(33)	36	23	30	(40)	39	19	21.5	6.9	2169
Pothohar	35	0	(100)	29	20	0	(100)	31	9	15.4	3.1	1418
LU 26S	76	14	(50)	56	21	26	(30)	41	23	27.6	9.4	1752
Pavar	23	4	(67)	50	23	14	(70)	29	12	16.2	5.4	1442
SA-75	51	8	(67)	55	24	24	(40)	33	18	15.3	5.3	1531
Indus-79	—	—	—	35	20	12	(70)	26	13	—	—	1637
LU60-1	—	—	—	47	22	26	(50)	41	18	—	—	—
Sandal	33	2	(83)	—	—	—	—	—	—	13.0	5.4	1351

R.S. = Relative shoot yield (% of control).
P = Plant.

Table 7. Relationship among the data from various studies

Relative shoot yield		*vs*	Relative shoot yield		Correlation coefficient
m*M* NaCl			m*M* NaCl		
200	(set-I)	*vs*	200	(set-II)	+0.531*
200	(set-I)	*vs*	150	(set-III)	+0.534*
200	(set-I)	*vs*	300	(set-III)	+0.605*
300	(set-II)	*vs*	300	(set-III)	+0.315
200	(set-II)	*vs*	150	(set-III)	+0.325
Visual scoring vs visual scoring					
400	(set-I)	*vs*	400	(set-II)	+0.682**
Plant mortality vs plant mortality					
400	(set-I)	*vs*	400	(set-II)	+0.667*
Soil beds (grain yield) vs other techniques					
Grain yield at ECe (dSm^{-1})			m*M* NaCl		
10		*vs*	200	(R.S. set-I)	+0.590*
20		*vs*	200	(R.S. set-I)	+0.716**
10		*vs*	150	(R.S. set-III)	+0.069
20		*vs*	300	(R.S. set-III)	+0.768**
10		*vs*	Yield in field		+0.208
20		*vs*	Visual scoring (set-I)		+0.690**
20		*vs*	Visual scoring (set-II)		+0.584*
20		*vs*	Plant mortality % (set-I)		−0.665*
20		*vs*	Plant mortality % (set-II)		−0.663*

* = Significant (P = 0.05)
** = Significant (P = 0.01)
R.S. = Relative shoot yield.

Table 8. Performance of nine wheat cultivars in saline nutrient medium (set-IV) and soil beds (set-II)

Cultivars/ tolerance groups	Salinity levels							
	250 m*M* NaCl			350 m*M* NaCl			ECe dSm^{-1}	
	Fresh shoot weight (g plant^{-1})		Plant mortality (%)	Fresh shoot weight (g plant^{-1})		Plant mortality (%)	10 Grain yield (g/6 plant)	20
Tolerant								
LU 26S	2.83 ± 0.72†	(43)‡	0	1.33 ± 0.70	(20)	17	15.56	3.82
Blue Silver	1.95 ± 0.71	(35)	0	1.11 ± 0.80	(20)	17	19.27	1.99
Lyp-73	1.50 ± 0.25	(37)	0	0.46 ± 0.60	(11)	40	15.90	0.75
Moderate								
Pak-81	1.34 ± 0.55	(20)	0	0.35 ± 0.40	(5)	60	14.20	1.16
Bah.-79	0.87 ± 0.13	(17)	40	0.00 + 0.00	(0)	100	7.96	0.00
SA-75	0.86 ± 0.56	(21)	20	0.24 ± 0.38	(6)	60	10.87	0.78
Sensitive								
Pothohar	1.08 ± 0.96	(20)	33	0.35 ± 0.41	(6)	50	12.46	0.14
Pb.-81	1.39 ± 0.44	(29)	0	0.14 ± 0.05	(3)	83	13.11	0.75
Indus-79	1.98 ± 0.68	(26)	0	0.36 ± 0.80	(5)	80	12.12	1.67

† Mean ± Standard deviation. P = Plant
‡ % of control
Correlation studies. Relative
Grain yield at 10 dSm^{-1} *vs* Fresh shoot wt. at 250 NaCl = +0.753**
Grain yield at 20 dSm^{-1} *vs* Fresh shoot wt. at 350 NaCl = +0.794**
Grain yield at 20 dSm^{-1} *vs* Plant mortality at 350 NaCl = −0.654*
* P = 0.05
** P = 0.01

visual scoring seemed to be associated with salt tolerance based on grain yield (see Tables 6–8).

Reproducibility of results

Fourteen cultivars were tested 3 times (twice in Faisalabad and once in Bangor, U.K.) to determine the relative salt tolerance of various cultivars. The data regarding the relative yields (per cent of the respective controls) at high salinity and their rating on this basis are included in Tables 5 and 6 along with the data for grain yield in soil beds and salt affected field as discussed later.

The results obtained in the three sets show close association (Table 7). Relative shoot yield of various cultivars at 200 mol m^{-3} NaCl salinity in set I was significantly and positively correlated with relative shoot yield at 200 mol m^{-3} NaCl (set II) and at 150 and 300 mol m^{-3} NaCl (set III) (coefficient of correlation values were +0.531, +0.534, and +0.605, respectively). Similarly, results based on visual scoring and plant mortality at 400 mM NaCl were significantly and positively correlated for sets I and II. The lack of significant correlation and some inconsistencies in the results in some instances were due to: (1) complex interaction between environmental factors and genetic make up of the species controlling salt tolerance mechanisms (humidity, temperature and day length varied in the 3 sets), (2) the level of salinity used in different sets, (3) the use of fresh weight instead of dry weight in set III as the selection criterion, and (4) the major intravarietal variations with respect to salt tolerance (see Rashid, 1986; Yeo and Flowers, 1984). Nevertheless, the varieties could be grouped into salt tolerance classes which would minimize these inconsistencies (Table 8).

Validity of results

Considering absolute grain yield obtained under semi-controlled conditions in artificially salinized soil beds as the valid test, the salt tolerance data at seedling stage (shoot weight) and the grain yield data from field experiment (24 replications) were compared against it (Table 6). The correlations

between screening data were statistically significant in many instances (see Table 7). These conclusions are further supported by the data given for another experiment conducted in Bangor under controlled conditions (Table 8). Here the correlations between grain yield (soil beds; Faisalabad) and fresh shoot weight (solution culture; Bangor) were significant. On the basis of ratings (Table 5) it is clear that the most tolerant and the most sensitive classes were represented by the same group of varieties in most instances. The inconsistent behaviour of some 'varieties' such as Bahawalpur-79, Chenab-79, Lyp-73 and Pb.-81 in different tests was largely due to the intravarietal variation with respect to salt tolerance. The salinity in the field was only moderate in most replications and showed great heterogeneity which rendered the positive correlations as non-significant.

Conclusions

For reasons discussed earlier, there seems to be no single flawless or universally applicable method for screening of wheat (or other crops) varieties for salt tolerance. Nevertheless, some workable comparisons are necessary to exploit the genotypic differences in salt tolerance of crops.

The following guidelines may be kept in view for this purpose: (1) the cultivars under test must grow under uniform conditions of nutrition, salt composition, total salinity, temperature, light *etc.*; tall and dwarf varieties should be widely spaced, (2) the cultivars should receive their own optimum requirements as far as possible; long duration and short duration cultivars should be tested separately, (3) screening period must include the critical stages of growth, (4) the conditions for selection should be as close to the field conditions as possible, (5) screening of a large number of cultivars in a short period should be possible.

Clearly, most decisions regarding the suitability of various aspects of the technique to be used would depend upon the local situation. The selection of experimental conditions such as temperature, humidity, type of salt, level of salinity, duration of salinity *etc.*, should match the soil, climate, and management practices of the tract. For example, a salt tolerant cultivar needs to be tolerant to high pH, high ESP and low Eh for successful growth in the saline-sodic and rather dense rice soils of Pakistan.

It may be suggested that screening at seedling stage is not only less laborious, less time consuming and less expensive, but has high reliability. Screening under natural field conditions is not feasible due to the high degree of soil heterogeneity. Other techniques such as regular flushing of relatively pervious fields with saline waters are definitely more demanding and only a limited number of genotypes could be handled. The view that 'the genotypes giving high yields under non-saline conditions would also give higher yields under saline field conditions because the salinity in the cultivated fields exists in patches of very low to moderate salinity', has yet to be tested although our observations do not support it.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 325–329.
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PLSO PN 171

A method for investigating the influence of soil water potential on yield and water use efficiency of some spring wheat cultivars

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Key words: harvest index, soil water potential, water use efficiency, wheat, yield

Abstract

A method is described for supplying greenhouse pots continuously and automatically with water while maintaining different soil water potentials. This system consists of diaphragm cells which are vertically inserted into the soil, storage water bottles, regulating manometers, electronic control equipment and a vacuum pump.

This method was used for investigating the influence of genotypical yield structure and temporally limited changes of water supply on the relation between yield and transpiration as well as water use efficiency of three spring wheat cultivars.

All three cultivars had an apparent ability to increase WUE as water supply decreased with only small differences in the WUE values between the cultivars. A positive correlation between harvest index and WUE was found.

Introduction

It is generally recognized that water supply has dominating influence on crop yields. Especially, a positive linear relationship has been established in the literature between total transpiration rate and dry matter yield of a number of crops (Arkley, 1963; Hanks, 1974; Sommer and Schwarz, 1985; de Wit, 1958). This relationship means that the maximum yield will be attained when soil water potential does not limit transpiration (Fig. 1).

Under the economical conditions of today, the aim cannot be the achievement of the highest possible yield, regardless of the level of inputs, but of low-input genotypes with a high yield stability. One of the most important inputs is the amount of water taken up by plant roots during specific time intervals and the total season, as well. Together with yield data it is possible to compare different cultivars due to water use efficiency (WUE). WUE has to be defined as

$$WUE = \frac{Y}{T}$$

where Y (g) is the economical yield and T (l) is the total seasonal transpiration.

Under field conditions evapotranspiration rates can be evaluated most precisely by using different types of lysimeters with confined soil volume representative of the surrounding soil (Pruitt and Lourence, 1985). An alternative to lysimeters for measuring evapotranspiration on field plots is the portable chamber technique (Reicosky, 1985). Both techniques allow reliable estimates of actual evapotranspiration which are necessary for water planning and irrigation scheduling.

To compare different cultivars due to WUE pot experiments under controlled conditions are of advantage. In pot experiments numerous soil and environmental parameters can be controlled. The goal of this paper is to describe a method suitable for investigating the influence of soil water potential on WUE of different spring wheat cultivars.

Method and procedure

The 'water comfort' (Hanks and Ashcroft, 1986) of plants does not depend as much on the quantity

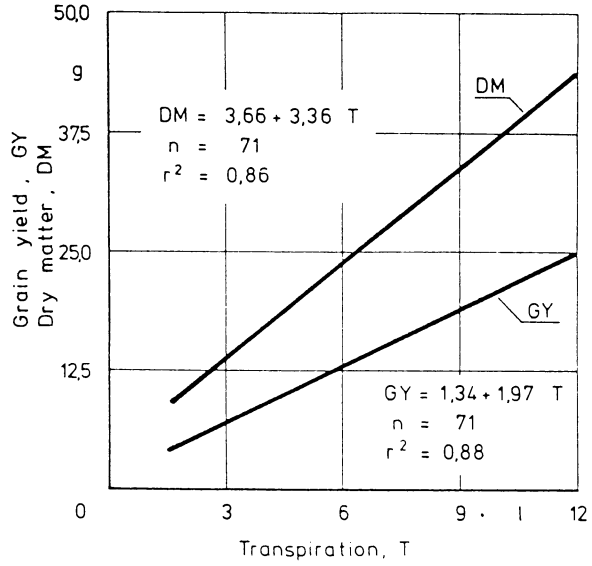

Fig. 1. The linear relationship between yield and transpiration rate.

of water present — soil water content — as it does on the water potential. Maintaining a wanted soil water potential during a pot experiment remains one of the most difficult technical problems (Slavik, 1974). Various types of so-called auto-irrigators have been proposed. Livingston (1918) used a porous tensiometer cup buried in the centre of the pot, distributing water sucked up from a reservoir. Today this device is commercially available for automatic irrigation of flowerpots. Double-walled pots with a porous inner wall have been used by some investigators, for example Hack (1971). The most simple soil potometer construction has been described by Lebedev and Solovev (1964). A sintered glass filter is the partition wall between the upper part of the potometer which is filled with soil and the water reservoir below. The amount of water taken up by the plants, which are growing in the soil, can be deduced from the changes of the water-table level in the potometric vessel. Braun and Schmidt (1972) have constructed special potometers for measuring the water consumption of trees. Czeratzki (1959) developed suction lysimeters which avoid the well-known oversaturation in the outflow zone of conventional lysimeters, by using horizontal diaphragm cells installed in the bottom of the lysimeters. Good results with these lysimeters led to the idea of water application to pots by capillary irrigation.

The demands on a pot technique for investigating the influence of soil water potential on the WUE are:

— Supplying small or large greenhouse pots continuously and automatically with water while maintaining different levels of soil water potentials ψ;
— Establishing a more even distribution of soil moisture than by other known methods during plant growth;
— Measuring of water uptake from different soil compartments with different soil physical conditions (soil water potential, soil density, soil structure).

Such a technique was first described by Sommer (1980/81) and Sommer and Tanasescu (1981). The

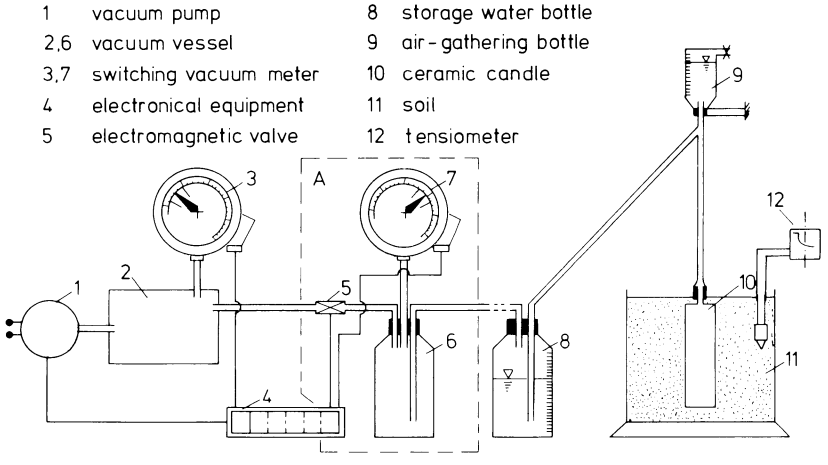

Fig. 2. The pot-technique for controlling soil water potential.

system consists of diaphragm cells which are vertically inserted into the soil, storage water bottles, regulating manometers, electronic control equipment and a vacuum pump (Fig. 2).

The vacuum pump (1) maintains a certain negative pressure which must be lower than the minimum soil water potential needed. This low pressure is controlled by a regulating vacuum gauge (3) and an electronic device. A unit A consists of an electromagnetic valve (5), a vacuum gauge (7) which is adjusted to the ψ wanted. By means of such a unit A several pots (11) which are connected in series can be supplied with water at a known water potential.

Water storage bottles (8) are connected with diaphragm cells (10) (Fig. 3), by means of water filled flexible tubes of 10 mm outside and 6 mm inside diameter. An air-collecting bottle (9) is interconnected to prevent interruptions of the water supply by air bubbles. The soil water potential (matric potential) is controlled by recording tensiometers (12).

Whenever the equilibrium in the upper part of the storage bottle (8) and the absolute value of the soil water potential ψ is impaired by water uptake of the plotted plants water will flow into the soil continuously. The amount of water supplied to the soil can be measured by means of a scale on the storage bottle (8).

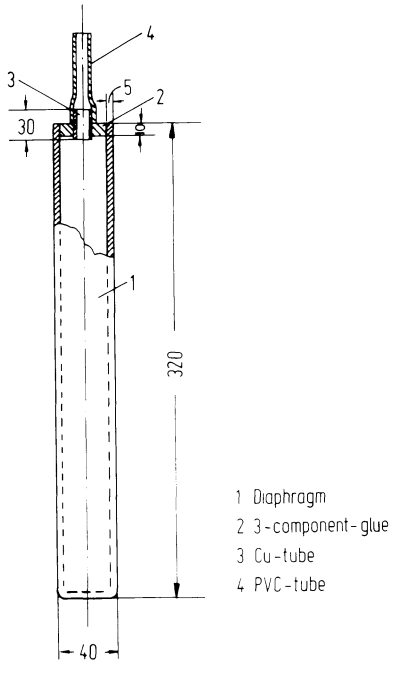

Fig. 3. The dimensions of the diaphragm cells used.

Ten treatments of water supply were used for investigating the influence of two different levels of soil water potential — maintaining during several growth stages — on WUE of spring wheat cultivars (Fig. 4).

A time interval is necessary to change the soil water potential from $\psi_1 = -70$ h Pa to $\psi_2 = -600$ h Pa or from ψ_2 to ψ_1 until the new equilibrium is reached.

Mitscherlich pots of 20 cm inside diameter and 22 cm height were used for single spring wheat plants. The pots were filled with a loamy soil. A vertical hole (about 50 mm wide) was drilled into the soil which was filled with a soil-water mixture and then the ceramic cell was pushed into the hole. In this way, a good contact between the cell and the soil is reached. The pots prepared in such a way were set upon movable greenhouse trollies in an air-conditioned greenhouse. The day/night air temperature in the greenhouse was 20/15°C and the relative air humidity 60%. Three spring wheat cultivars (*Triticum aestivum*) were compared in two repetitions of the same experiment: cv. 'Selpek', cv. 'Archill' and cv. 'Sokrates'. Six growth stages were taken into account: emergence, beginning of tillering, shooting, heading, end of flowering and ripeness (Fig. 4).

Results

In the following two figures the representative results of the one experiment are shown. For the three cultivars yield and transpiration were closely linearly correlated over all water supply treatments with almost identical yield-transpiration regressions (Fig. 5).

Harvest index, the ratio of grain yield to total plant weight, decreased with increasing transpiration, but this relationship was significant only for 'Achill' and 'Selpek'.

Figure 6 shows water use efficiency (WUE) as a function of transpiration and grain yield, respectively.

All three cultivars had an apparent ability to increase WUE as water supply decreased. The water supply treatments differed considerably in their water use efficiency values, but there were only

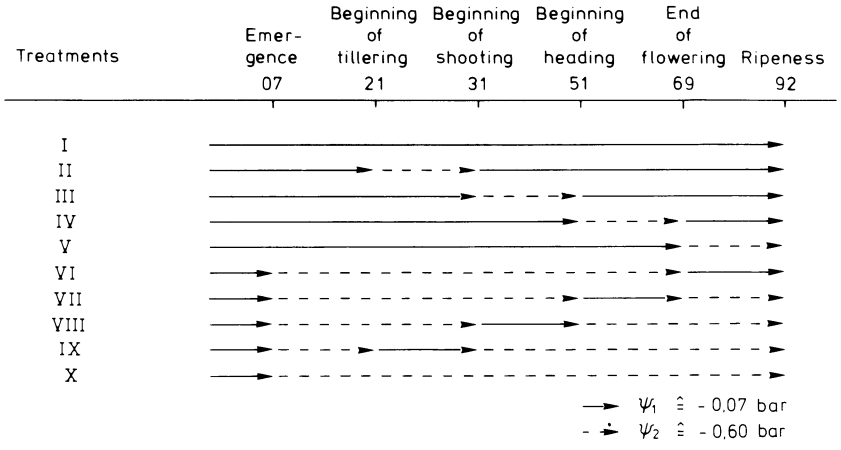

Fig. 4. 10 treatments of water supply used (Schwarz, 1987).

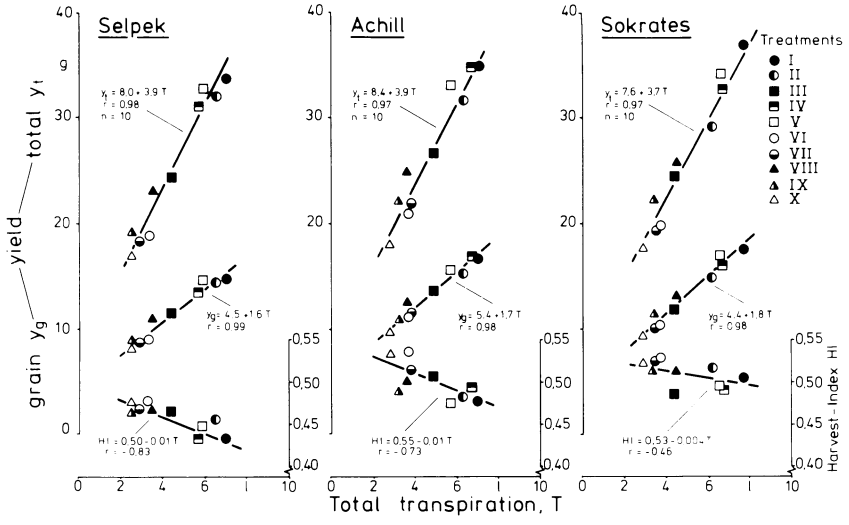

Fig. 5. Total yield, grain yield and harvest index as a function of transpiration for three spring wheat cultivars (Schwarz, 1987).

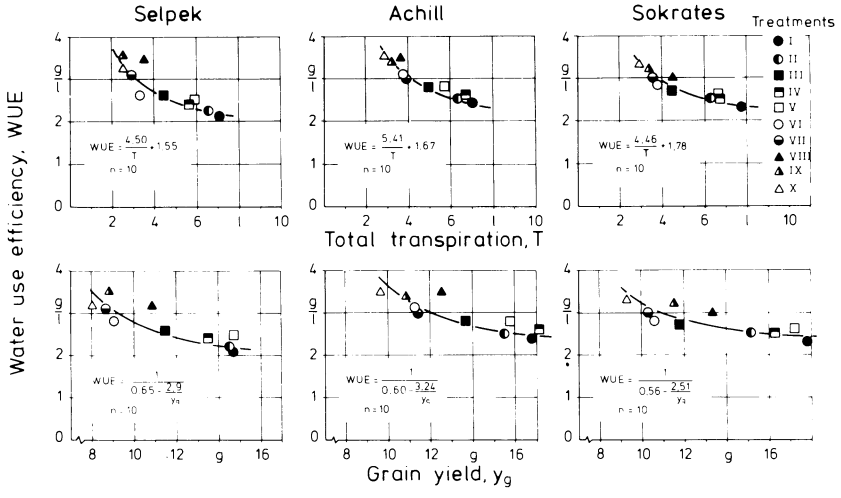

Fig. 6. Water use efficiency as a function of total transpiration and grain yield for three spring wheat cultivars (Schwarz, 1987).

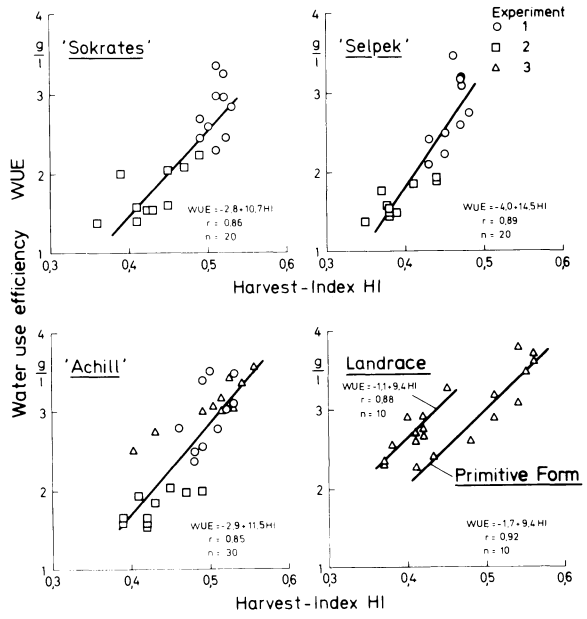

Fig. 7. Water use efficiency as a function of harvest index (Schwarz, 1987).

small differences between the cultivars. These results referred to an only small genetic variability.

In a further experiment a landrace and a primitive form were compared with 'Achill' instead of the cultivars 'Selpek' and 'Sokrates'. Similar relationships as just described were found.

The positive linear regression between total yield and grain yield, respectively, and transpiration referred to a positive relationship between water use efficiency and harvest-index, as it is shown in Figure 7. That means that the harvest-index is a very important parameter to increase WUE by breeding programmes.

Conclusions

In principle the described method is suitable for investigating the influence of soil water potential on yield and water use efficiency. Recently, the technique was developed further on by using modern tensiometers for regulating the soil water potential. This includes a computer controlled data collection system.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 331–337.
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PLSO PN172

Water potential as a selection criterion for drought tolerance by different durum wheat genotypes

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Key words: drought tolerance, osmotic potential, pre-dawn potential, *Triticum durum*

Abstract

Twelve durum wheat cultivars (*Triticum durum* desf.) originated from different countries (Ethiopia, Austria and Italy) were sown in pot culture, then grown under drought stress and normal conditions. Drought stress was imposed temporarily for 7–10 days starting at booting stage.

Leaf osmotic potential was determined by using the Pressure-Volume-Curve technique. Five genotypes with high and low osmotic adjustment were selected for further studies. The difference in osmotic potential between the varieties were largely confirmed. These varieties were intercrossed and the osmotic potential for parents and F_1 were measured. The differences between varieties could be mostly confirmed.

The same cultivars grown in pot culture under three different moisture levels, 20%, 50% and 80% field capacity, have shown different trends in their osmotic potential. Cullivars which have had low osmotic adjustment under short stress period have shown better osmotic adjustment under 20% soil water content. This leads to the conclusion that the effect of drought depends on the cultivar and the extent of stress period. Genotypes with high osmoregulation tend to be high yielding. On one hand grain yield from plants grown under 20% soil water content was low, as should be expected. On the other hand, plants grown under 50% and 80% soil water content yielded nearly the same. Some genotypes at 50% soil water content outyielded those grown at 80% soil water content.

Introduction

Environmental conditions are decisive in achieving potential yield production of world crops. Adequate water supply makes a great contribution to the performance of field crops. For the majority of these plants shortage of water supply constitutes the most important limiting factor especially in arid and semi-arid cultivation areas.

According to Dudal (1976) 26% of world's land utilization for crop production is limited by drought stress. In breeding programmes it is preferable to pursue more simply measured yield-correlated characters to be applied as selection criteria in early segregating generations. Any physiological attributes correlated to low water supply might be important and effective characteristics for breeding field crops in dry regions. Out of all physiological variables studied, osmoregulation was suggested as a possible selection criterion for grain yield where drought stress is a limiting factor (Blum and Sullivan, 1986; Morgan, 1983).

Osmoregulation was found to be positively correlated with average rainfall. Under irrigation, grain yield of wheat was positively associated with leaf water potential, while non-irrigated plants showed only weak insignificant positive association (Fischer and Wood, 1979). Blum *et al.* (1983) pointed out a positive association between yield and osmoregulation of some wheat cultivars subjected to high osmotic stress in solution culture under glasshouse conditions.

Westegate and Boyer (1985) reported negative effects of low water potential during early stages of grain development in maize on the number and weight of kernels produced. However, during late grain development, low water potential affected grain weight drastically. Namuco and O'Toole (1986) found a linear relationship between water potential of rice leaves and the percentage of fertile spikelets. An association between yield and osmoregulation of some sorghum varieties has been also reported by Abdussalam (1986).

Genetic variation of the mechanisms of osmoregulation has been revealed and described in several reviews, *e.g.*: Morgan (1977) pointed out differences in osmoregulation between wheat genotypes and discussed its positive effect on yield achieved. Morgan *et al.* (1986) studied genetic variation in osmoregulation in bread and durum wheats and found that genotypes with high osmoregulation achieved 11–17% higher yield in bread wheats and 7% more in durum wheats, than those with low osmoregulation. The authors reported evidence favouring glasshouse measurements of osmoregulation as an early selection criterion in wheat breeding programmes.

The present study addresses itself to the following questions:

(a) Are there differences in drought resistance between durum wheat cultivars from different geographical regions?
(b) Are there common attributes to drought resistance among investigated genotypes?
(c) Can these physiological attributes, *i.e.*, osmotic potential (osmoregulation), pre-dawn potential, be used as selection criteria for yield performance in early generations in breeding programmes?

Materials and methods

Durum wheat varieties (*Triticum turgidum* ssp. *durum* Desf.) originated from different geographical regions, *i.e.*: Ethiopia, Austria and Italy were used for the investigations. Plants were raised in 20-cm-diameter pots under glasshouse and greenhouse conditions to determine leaf water potential and osmotic potential of the different varieties. Pots were filled with about 2 kg in volume ratio of 1/2 compost: 1/4 pot culture substrate: and 1/4 peat moss mixture. For glasshouse trial 2 pots each with 20 kernels were planted per variety. These were thinned later to 10–12 plants per pot. Just after germination the pots were placed in a cool room at 4°C for 2 weeks to allow vernalisation. Subsequently they were placed for another week at 10°C to induce tillering. Temperature was then raised gradually till it reached 20°C/15°C for day/night temperature respectively. A radium bulb of 400 W ensured a light intensity supply of 12–14 Klux for a period of 10 hours daily. After 3 weeks this photoperiod was extended to 14 hours between 5 and 19 hrs. A liquid fertilizer (Wuchsal = 10 : 10 : 7) was applied to maintain proper plant growth. Controlling powdery mildew necessitated spraying with different kinds of fungicides during the whole vegetation period until booting. Flag leaves of primary tillers were sampled at booting stage as the plants were droughted over a period of 8 days on one pot while the other pot was treated normally. Leaf water potential was then determined for both stressed and non-stressed plants.

For the greenhouse trial, the field capacity (FC) of the soil mixture was determined, nine pots per genotype were then filled with the soil, planted seedlings were then vernalised. Three weeks after germination, the pots were placed in the form of a complete block design with 3 replicates. Per replicate and genotype three different treatments of field capacity, *i.e.*, 20%, 50% and 80%, were practiced and kept constant by weight during the whole vegetation period, until full development of the flag leaves. The increase in plant weight during this period was always considered and compensated.

Measurements

A commercial pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, Cal., USA) was used in this investigation to achieve reliable field measurements of leaf water potential. Full developed flag leaves were harvested and kept with their excised ends in glass cylinder containing water and covered with parafilm (polyethylene sheet) for 24 hours in the dark at

room temperature of 20°C. This allowed leaf rehydration and full recovery from water stress. The hydrated leaf was weighed and inserted in the pressure chamber where leaf water potential was determined. By free transpiration, the leaf was allowed to dehydrate and its weight was then scored before being reinserted in the pressure chamber. This procedure was repeated frequently until enough measurements were recorded. Leaf fresh weight (FW) was recorded in correspondence with leaf water potential. Dry weight (DW) was achieved by oven drying at 100°C for 24 hours.

Relative water content (RWC) was calculated by using the formula:

$$RWC = \frac{FW - DW}{SW - DW}$$

where SW corresponds to leaf saturated (hydrated) weight.

Leaf water potential and the corresponding value of water saturation deficit (WSD) were then plotted on a chart as a type II transformation:

ψ_t^{-1} *vs* WSD after Tyree and Richter, 1981.

$$WSD\ \% = 100\,(1 - RWC)$$

After turgor loss point, ψ_t^{-1} *vs* WSD shows a linear relationship. By extrapolating this line, the osmotic potential at full saturation is determined by eye, eventually calculated for the Y-intercept.

The difference between the total leaf water potential and osmotic potential gives the turgor potential (ψ_p) before the leaf reaches the turgor loss point.

Results

Pre-dawn potential

Pre-dawn water potential for the investigated durum wheat varieties under the given field capacities (Table 1) reveals significant differences between cultivars as well as between water stress treatments.

The results presented in Table 1 show that a long-term drought condition (20% FC) expressed itself noticeably all over the investigated genotypes. The achieved values varied from −0.64 to −1.19 MPa with a mean pre-dawn potential of -0.96 ± 0.10. The other two treatments obtained similar results and were not significantly different from each other. The differences between 20% and 50% field capacity were revealed and statistically assured.

Table 1. Pre-dawn water potential (−MPa) for 5 durum wheat varieties grown under different water field capacities

Variety	Water field capacity (%)		
	20	50	80
Boohai	0.64	0.65	0.15
DZ-04-688	1.09	0.85	1.01
Attila	1.19	0.15	0.56
Unidur	1.07	0.19	0.33
Valgerardo	0.79	0.30	0.44
$\bar{X} \pm s_{\bar{x}}$	0.96 ± 0.10	0.43 ± 0.14	0.50 ± 0.14
calculated t value	3.08*		
tabulated t value	2.67		
5%	2.78		
1%	4.60		

Under short periods of drought stress (Table 2) the pre-dawn potential of the different genotypes varied from −1.08 to −2.63 MPa with a mean value of -1.97 ± 0.22 and differed significantly from that of unstressed plants.

The results of Table 2 confirmed the differences between genotypes in regard to their reac-

Table 2. Pre-dawn potential (−MPa) of 8 durum wheat genotypes under short-term stress conditions in comparison with unstressed plants

Genotypes	Pre-dawn potential (−MPa)	
	Stressed	Unstressed
Boohai	1.08	0.56
DZ-04-688	2.63	0.21
Attila	1.20	0.75
Unidur	1.89	0.20
Valgerardo	2.62	0.51
Boohai × DZ-04-688	2.54	0.44
Boohai × Valgerardo	1.95	0.25
Attila × Valgerardo	1.83	0.30
$\bar{X} \pm s_{\bar{x}}$	1.97 ± 0.22	0.40 ± 0.07
calculated t value	6.80***	
tabulated t value		
at 5% threshold	2.36	
at 1% threshold	3.50	

tion to drought stress. Clear differences in pre-dawn potential between short-term stressed plants and the unstressed ones are demonstrated. Generally speaking, with the exception of few varieties, a high pre-dawn potential has been obtained under long-term stress conditions than in the case of short term drought stress.

Osmotic potential

The osmotic potential of different durum wheat varieties under the different water regimes (Table 3) could also reveal obvious differences among cultivars as well as among the water treatments.

Under 20% water field capacity the ψ_0 ranged between -1.54 and -1.78 MPa with a mean value of -1.67 ± 0.04. The difference between this treatment on one side and the other two variantes on the other side was statistically highly significant. The achieved values of ψ_0 under 50% water field capacity varied between -1.08 and -1.47 with a mean of -1.30 ± 0.07, while under 80% it ranged between -1.11 and -1.47 with a mean value of -1.28 ± 0.06 MPa.

These genotypes demonstrated clear variability in ψ_0 under short-term drought stress (Table 4). The results differ from those obtained under long-term stress treatment.

No significant difference was shown to exist between stressed and unstressed plants in regard to their osmotic potentials. Under short-term stress conditions the ψ_0 varied between -1.35 and -1.66 MPa with a mean value of -1.45 ± 0.04. Unstressed plants achieved a mean value of -1.30 ± 0.05, with a wide range of variation between -1.08 and -1.49.

The response of different genotypes to drought stress was obviously affected by the length of the stress period. The Ethiopian variety DZ-04-688 which has shown poor osmoregulation under short-term water stress, could achieve somewhat better osmotic adjustment under long-term drought period.

Table 3. Osmotic potential (−MPa) of 5 durum wheat varieties in relation to different water field capacity

Variety	Osmotic Potential (−MPa) Water field capacity		
	20%	50%	80%
Boohai	1.70	1.47	1.47
DZ-04-688	1.54	1.40	1.22
Attila	1.64	1.08	1.11
Unidur	1.77	1.28	1.31
Valgerardo	1.78	1.25	1.27
$\bar{X}$	1.67	1.30	1.28
$s_{\bar{x}}$	0.04	0.07	0.06
calculated t value	4.81**		
		5.42**	
tabulated t value			
5% threshold	2.78		
1% threshold	4.60		

Table 4. Osmotic potential (ψ_0 in −MPa) of eight durum genotypes under short-term stress conditions in comparison with unstressed plants

Genotypes	ψ_0 in (−MPa)	
	Stressed	Unstressed
Boohai	1.40	1.21
DZ-04-688	1.42	1.45
Attila	1.37	1.36
Unidur	1.57	1.08
Valgerardo	1.36	1.28
Boohai × DZ-04-688	1.46	1.37
Boohai × Valgerardo	1.66	1.49
Attila × Valgerardo	1.35	1.14
$\bar{X}$	1.45	1.30
$s_{\bar{x}}$	0.04	0.05
calculated t value	2.34	
tabulated t value		
5% threshold	2.36	

Kernel weight per main spike

The main purpose of these experiments is to focus on osmoregulation measurements and to examine the possibility of using them as an early generation selection criterion for high yield in breeding programmes for water-limited regions. The yield achieved by the main spike of these varieties under the three water regimes is presented in Table 5.

Under greenhouse conditions and in pot trials it is clear that the absolute yield obtained could not be used directly to evaluate the yield potential of the varieties under consideration, whereas it gave the possibility to compare these varieties

Table 5. Yields obtained in g per main spike from different varieties under different water yield capacity

Variety	Water field capacity (%)		
	20	50	80
Boohai	0.63	0.74	0.78
DZ-04-688	0.61	0.85	0.73
Attila	0.79	0.87	0.77
Unidur	0.34	0.52	0.56
Valgerardo	0.57	0.80	0.90
$\bar{X}$	0.59	0.76	0.75
$s_{\bar{x}}$	0.07	0.06	0.05
t value	1.89 ns		
		1.78 ns	
t 5%	2.78		

Table 6. Yields obtained in g per main spike from different genotypes from stressed and non-stressed treatment

Genotype	Stressed	Nonstressed
Boohai	0.55	0.67
DZ-04-688	0.57	0.69
Attila	0.99	1.16
Unidur	0.61	0.67
Valgerardo	0.79	0.84
Boohai × DZ-04-688	0.92	1.18
Boohai × Valgerardo	0.94	0.96
Attila × Valgerardo	1.18	1.24
$\bar{X}$	0.82	0.93
$s_{\bar{x}}$	0.08	0.08
t value	1.0 ns	
t 5%	2.36	

under the given circumstances. As it is clear from the presented data, the kernel weight per main spike is generally low. An increase in kernel weight was found to be related to increasing water supply. However, the differences between the three treatments could not be statistically ensured, whereas, a clear association between water field capacity and yield of durum wheat plants could be revealed. Within each treatment, the varieties differed obviously in their yield performance. Significant differences between the varieties were statistically assured. The kernel weight under 20% water field capacity ranged between 0.34 and 0.79 g with a mean value of 0.59 ± 0.07 g. On the other hand, the yield achieved under 50% and 80% varied between 0.52 and 0.87 with a mean value of 0.76 ± 0.06 and 0.56–0.90 with a mean value of 0.75 ± 0.05 respectively.

The Austrian variety Unidur differed significantly from all other cultivars under consideration and obtained the lowest yield under the three water regimes. The other Austrian variety Attila maintained its yield more or at a less stable level although a detectable decrease under 80% water field capacity was recognized. The Ethiopian varieties showed slight differences in their response to increasing water supply. Boohai yield increased with the increase of water field capacity, whereas DZ-04-688 reached its optimum under 50% treatment, but the yield dropped significantly under 80% water field capacity behaving similarly to Attila. On the other hand, Valgerardo, the Italian demonstrated an obvious positive association between yield and water supply under the described circumstances.

Short-term stress treatment affected the investigated varieties differently. Droughting the plants at booting stage decreased the yield (Table 6). Normal irrigated plants achieved higher yield than those under water stress conditions. Differences between genotypes within each treatment were obvious than those between treatments. Kernelweight per main spike under stress condition ranged between 0.55 and 1.18 g with a mean value of 0.82 ± 0.08 g. On the other hand, it varied between 0.67 and 1.24 g with a mean value of 0.93 ± 0.08 gr under normal water supply, whereby F1-hybrid plants (Attila × Valgerardo) achieved the highest yield values mentioned in both cases and thereby overyielded parental types. These differences between investigated genotypes were ensured statistically while those between stress treatments could not be statistically revealed.

The investigated cultivars were also sown under field conditions and the kernel weight per main spike is presented in Table 7. The yield achieved was 3 to 5 times higher than that obtained under greenhouse conditions in pot trials. These five cultivars could be grouped into 3 significantly different categories: high-yielding cultivars (Valgerardo and Boohai), middle-class yielding type (Attila) and low yielding cultivars as Unidur and DZ-04-688. Under short- and long-term water stress treatments, each of these cultivars reacted differently.

Table 7. Kernel weight in g obtained from potted plants under 50% water field capacity, glasshouse and field conditions

Variety	50%		Glasshouse		Field	
	Absolute	Relative	Absolute	Relative	Condition	DMT[a]
Boohai	0.74	33.18	0.67	30.04	2.23	ab
DZ-04-688	0.85	62.04	0.69	50.36	1.37	c
Attila	0.87	43.07	1.16	57.42	2.02	b
Unidur	0.52	35.37	0.67	47.18	1.47	c
Valgerardo	0.80	32.78	0.84	34.42	2.44	a
$\bar{X}$	0.76		0.81		1.91	
$s_{\bar{x}}$	0.05		0.07		0.16	
		0.55				
			6.76**			
t-value				6.47**		
t 5% =	2.78					
t 1% =	4.60					

[a]Duncan Multiple Range Test

Discussion

Under normal field conditions, these cultivars could be differentiated according to their yield performance. The yield achieved tended to be positively associated with the osmotic potential. High-yielding cultivars were distinguished by high osmotic adjustment. These findings confirm what have been already recorded by Abdussalam, 1986; Blum *et al.*, 1983; Fischer and Wood, 1979; Morgan *et al.*, 1986.

Potted plants under controlled climatic conditions, *i.e.*, glass or greenhouse, differed from field-grown plants in their water relations as had been reported by Slatyer (1963). This could clarify the differences in yield achievement between pot plants in glasshouse and field-grown plants (Table 7). In spite of that, the differences in osmoregulations between the cultivars under glasshouse conditions were obvious and correlated well with field performance, that useful information could be concluded from a series of reliable measurements. According to the results, it seems possible to screen germplasm for osmoregulation by using potted plants under greenhouse conditions. As a selection criterion at the beginning of a breeding programme, where drought stress is a limiting factor, osmoregulation was also recommended by Morgan (1983), whereby negative screening for poor osmoregulation could be more reliable and useful in elininating drought sensitive plants.

Plants could achieve better osmoregulation under long-term stress than under short period droughting, though genotypical differences occurred and should be also considered. Some varieties can adapt themselves well to long-term stress conditions and achieve reasonable yield. Such transposition seems unrealisable under short-term stress treatments. This confirms the results obtained by Vilela and Moraes (1986).

Significant differences could be scored between the genotypes used in regard to their pre-dawn potential and also to osmotic potential. A positive association between osmotic potential and yield was revealed under long-term as well as short-period droughting conditions. On the other hand, neither a significant association nor a clear tendency could be assigned for pre-dawn potential and yield performance under all described conditions. This confirms the usefullness of osmotic potential as a possible selection criterion for drought tolerance where water supply is a limiting production factor (in arid and semi-arid regions) as was suggested by Morgan (1983), Blum and Sullivan (1986). In breeding programmes for normal conditions, osmotic adjustment could be a good tool in screening for high yield.

In general, the osmotic potential seems to be an indicator rather than a determinant factor for yield potential under stress conditions. The fact that some varieties yielded less when droughted, *i.e.* Valgerardo, inspite of their high osmotic adjustment, may lead to the conclusion that the osmoregulation functions at the cost of the yield and that the effect of other factors contributing to yield performance should be also considered.

Acknowledgement

The authors express their gratitude to Prof. Hanno Richter and Dr S Kikuta for their indispensable help and valuable scientific comments, proposals and advice, also to the Austrian Foreign Students Exchange Service for the financial support.

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PLSO PN 173

Application of *in vitro* techniques for screening plant genetic variability

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Key words: aluminium, cowpea, *in vitro* techniques, manganese, potato, salt, screening, soybean

Abstract

Tissue culture can be used to select the most desirable genotypes from large numbers of variants which exist in nature. Genotypes of potato show considerable differences in growth responses to saline conditions. Two varieties (Hansa and Frühbote) and three wild species (*S. chacoense, S. phureja, S. sparsipilum*) were tested for their tolerance or sensitivity to salt. The data show very clearly that 'Frühbote' is well able to tolerate salt, but that 'Hansa' is extremely sensitive to it. The three wild species lie between them in salt tolerance.

The calli of two different cowpea genotypes were cultured on media with different manganese concentrations. Tvu 91 may be seen as a very sensitive genotype, which produced no calli, even with 50 m*M* manganese in the medium. Tvu 1897, in contrast, produced a callus even at the highest concentration of manganese.

There is evidence for the inheritance of tolerance to aluminium in the soybean. Twelve soybean varieties with a known sensitivity to aluminium were tested. The variety 'Ronda' is very tolerant to aluminium and 'Effi' is very sensitive. 'Maple Arrow' is a variety which is between 'Ronda' and 'Effi' and showed this position very clearly with the different concentrations of aluminium.

Introduction

For many centuries farmers used a trial and error approach in selecting the crops which they could grow with success. They chose those which grew well in the existing climatic and field conditions. With the industrial revolution there came a great increase in the demand for varieties with a higher yield. A profession developed of specialist breeders, who were able to develop strains with genetic uniformity to give even greater yields when conditions were right.

But the impact of fluctuating environmental conditions became increasingly obvious. Consequently, interest became more and more concentrated on the importance of yield stability despite changing or extreme environmental conditions.

Increased production must come either from higher yields on existing arable land, or from the opening of less fruitful areas to the growing of crops. There are a number of methods available to help to reduce the deleterious influence of a harsh environment on crop production. These methods are mainly concerned with soil conditions, since climatic factors seldom lend themselves to positive change.

The most promising solution seems to be the possiblity of growing varieties or genotypes which have been specially adapted to resist particular stress factors. Therefore the emphasis has been laid firstly on defining the selection criteria, secondly on the screening of germplasm collections. Many methods of selection are required to examine the available species of plants and find those with the desired characteristics. The usual methods are based on selection from whole plants. These methods are dependent mainly upon growing plants in the field.

There have been some remarkable successes, but on the whole the methods are very time and labour consuming. The breeders are consequently ready to use any method which can simplify and reduce the

cost of the selection process. Many new methods have already been used to some extent, including cell and tissue culture. Perhaps gene recovery and transfer may create new opportunities in the future.

Plant tissue culture techniques were originally developed for the study of basic problems of nutrition and morphogenesis and they have led to a large number of important advances in these fields. In the past few decades, as the methods and the culture media have improved, so it has become clear that these techniques can also be applied to other aspects of plant science. For instance, the techniques have been recognized as bringing about a great improvement in plant breeding work.

Tissue culture can be used to select the most desirable genotypes from large numbers of variants which exist in nature. Many articles have been written about the possible contribution to agriculture of these methods, including that of spontaneous mutations selected through tissue culture (Widholm, 1976).

Three important limitations should be kept in mind while using tissue culture techniques:

1. A variant that appears in the tissue or in the cell while it is in culture, may be due to an inherited modification which occurs only in tissue culture, or to an epigenetic change which results from the effect of stress caused by the culture itself.

2. Selection through tissue culture techniques cannot be applied to processes that take place only on the differentiated multicellular level. Such processes can only be identified by screening the whole plant.

3. Cytological and nuclear changes together with the progressive loss of totipotency frequently occur in culture (Tal, 1983).

Apart form these limitations, many of the available *in vitro* techniques have been developed for only a small number of species compared to the vast number of crops which grow under different environmental conditions. Therefore much research work is necessary to extend the list of species which have been improved by such techniques in their capacity of producing food and other important products.

Despite these limitations, it is true that a successful programme for improving plants in general cannot work without the *in vitro* techniques for genetic and physiological studies, including screening for desired characteristics.

Results and discussion

Screening for salt tolerance

Genotypes within many crop species show considerable differences in growth responses to saline conditions (Jones *et al.*, 1984). Selecting the survivors grown in a saline environment can either be done by growing crop plants in salinized soil or by irrigating the fields or the greenhouse with salinized water. All genotypes of potatoes are well known as being particularly sensitive to salt.

Two varieties (Hansa and Frühbote) and three wild species (*S. chacoense, S. phureja, S. sparsipilum*) were tested for their tolerance or sensitivity to salt. Nodal segments of five plants of each clone were transferred into nutrient medium supplemented with three different salt concentrations (4, 8 and 16 mmhos/cm).

The salt compounds and concentrations are given in Table 1. NaCl and $MgCl_2$ were present in a ratio of 70:30, because most salty soils contain sodium chloride and magnesium chloride. These nodal cultures were cultivated for 6 weeks at 20–22°C with a length of day of 16 hours. After 6 weeks the cultures were assayed according to 8 different criteria (for example the length of the shoots and roots and the fresh weight) in order to discover which criteria showed most clearly the differences between the clones.

Table 2 shows the relative length of the shoots and roots of the five clones grown on the saline medium. The data show very clearly that 'Frühbote' is well able to tolerate salt, but that 'Hansa' is extremely sensitive to it. The three wild species lie between them in salt toleration, with *S. phureja* the most sensitive and *S. sparsipilum* the most tolerant of the three (Arslan *et al.*, 1987).

These results were able to to be confirmed in experiments in salinized soil. The conclusion may

Table 1. The salt compounds and their concentration with reference to their tolerance to salt

Compounds added to the medium	Concentration (mmhos/cm)		
		8	16
NaCl	1.64	3.27	6.60
$MgCl \times 6H_2O$	0.57	1.14	2.35

Table 2. The influence of salinity on the length of shoots and roots of five potato genotypes

Salt concentration (mmhos/cm)		'Frühbote'	*S. sparsipilum*	*S. chacoense*	*S. phureja*	'Hansa'
0	shoot	100	100	100	100	100%
	root	100	100	100	100	100%
4	shoot	74.9	56.4	61.4	38.9	36.3
	root	82.6	92.4	43.2	39.2	40.2
8	shoot	59.6	58.5	48.0	28.0	19.2
	root	100.9	65.2	51.1	29.4	18.8
16	shoot	24.3	17.4	16.6	11.2	8.2
	root	58.8	32.5	24.2	2.3	0.9

be made that the *in vitro* method is applicable to screening for salt tolerance.

Screening for manganese tolerance

The cowpea (*Vigna unguiculata*) is a most important crop for the savannah of West Africa, as well as for other areas, but high manganese concentrations in the soil either prevent high yields or hinders growth to a great extent.

Consequently, an attempt was made to provide the breeders with genotypes which were tolerant to manganese, by developing an *in vitro* screening technique. When manganese is applied to the petioles of fully expanded leaves, it shows the same toxic symptoms on the leaf blades as if manganese had been absorbed by the roots.

Therefore, the attempt was made to find out whether a callus culture from leaf tissue could provide a possible screening system. The seeds of the four genotypes tested were germinated *in vitro* and entire plants were grown from them. Fully developed leaves were cut into segments 2–3 cm before removing the leaf veins. The most important factor for a good production of calli on the leaf tissue was the particular medium used (Mix, 1987).

The calli induced on the leaf tissue were put into suspension to provide a large amount of calli for the screening test. The calli were then plated on a medium on which all the genotypes had shown a very similar callus growth rate.

Different concentrations of manganese had previously been added to the medium. After about 6 weeks of cultivation, the cultures could be assayed. The result of callus growth rate with different concentrations of manganese in the medium may be seen in Figure 1.

Tvu 91 may be seen as a very sensitive genotype,

genotypes	10 µM	1 mM	10 mM	50 mM Mn
Tvu 91	18	8	1	-
Tvu 1897	25	25	4	1

Fig. 1. Callus formation in media with different manganese concentrations

which produced no calli, with 50 m*M* manganese in the medium. Tvu 1897, in contrast, produced a callus even at the highest concentration of manganese. The results *in vitro* confirm the known tolerance to manganese of these genotypes.

Consequently, it appears that this method is useful in selecting manganese tolerant cowpea genotypes as a first step. It has yet to be proved if it is possible to transfer this method with similar results to other species.

Screening for aluminium tolerance

The soybean is one of the most important oil and protein plants and the best growing areas are in the subtropics, where it is wet all the year round. There is evidence for the inheritance of tolerance to aluminium in the soybean and this natural genetic potential could be used to breed varieties which could accommodate themselves to tropical soils which are rich in aluminium for many reasons. In order to breed varieties with the required high tolerance to aluminium, it is necessary to develop a method to identify and select plants with the desired characteristics for future production (Meredith, 1978). This method must both be simple in application and able to give swift results, so that large numbers of genotypes can be thoroughly tested. Different methods of screening for aluminium tolerance have already been developed.

It is known that the toxic effects of aluminium reduce cell division activity in the root-tip meristem. Screening for aluminium tolerance can therefore be done on the roots. The aim was to develop an *in vitro* screening method. The experiments were made with twelve soybean varieties with a known sensitivity to aluminium.

The seeds of the varieties were germinated on filterpaper in Petri dishes. When the roots had reached a length of about 10 cm, the part at the root end without secondary root growth was cut off. This part was cut into segments of 1–2 cm, and was cultivated in a root inducing liquid medium in the dark at a temperature of 26°C.

The upper half of Figure 2 shows very clearly that it will hardly be possible to develop a medium which is optimal for all varieties or genotypes. In the lower half of Figure 2 it is easy to see the variation in the length of the secondary roots of the individual varieties caused by the different aluminium concentrations added to the medium.

The variety 'Ronda' is very tolerant to aluminium. In all the control tests, it had the smallest length of root growth. In contrast, 'Effi' is very sensitive to aluminium, but in the control tests always had the best length of root growth. But 'Ronda' grew the longest secondary roots in all the tests with aluminium, while 'Effi's' roots became progressively shorter as the aluminium concentration in the medium became higher.

'Maple Arrow' is a variety which is between 'Ronda' and 'Effi' and showed this position very

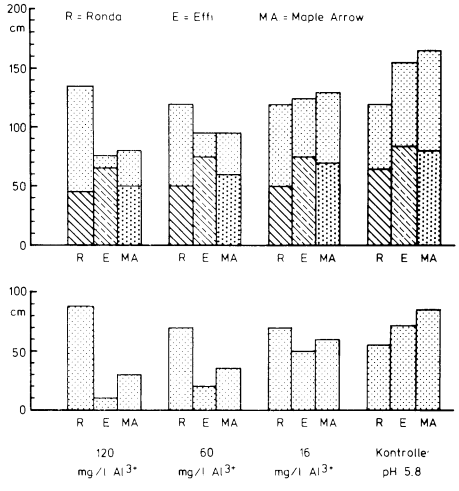

Fig. 2. Influence of different aluminium concentrations on root elongation

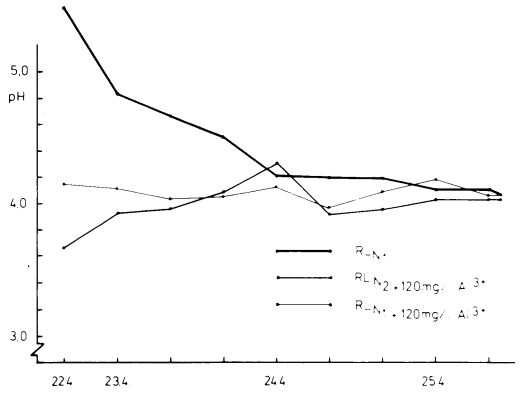

Fig. 3. The pH-curve during aluminium treatment of the root segments of the variety 'Effi'

clearly with the different concentrations of aluminium. Before these results could be achieved, the problem of the stability of the pH value during the period of cultivation of the screening had to be solved.

Each aluminium screening test was to be done with a pH value of 4.0, otherwise the aluminium loses its effect on the root tissue. Figure 3 shows the fluctuation of the pH values during an aluminium treatment of the root segments of the variety 'Effi'.

The fluctuation of the pH value of the culture could be prevented by the correct choice of the nitrogen compounds added to the medium. Figure 4 shows the influence of different nitrogen compounds on the stability of the pH value. Glutamine ($300\,mg^{-1}$) and nitrate ($500\,mg^{-1}$ $CaNO_3$) were equally well able to stabilize the pH value in the medium.

Glutamine, however, unfortunately greatly hindered growth in length of secondary roots. Apart from the stabilizing of the pH value, it is also necessary for the screening to test the appropriate aluminium concentrations. The testing of different concentrations (16–120 mg L Al^{3+}) showed clearly that the ideal test area was within the limits of an aluminium concentration of 20–60 mg L Al^{3+} with a time of treatment of two to three weeks.

The value of a screening method depends on its duration. The available information is confirmed in Figure 5, which shows that 'Ronda' is very tolerant to aluminium, that 'Effi' is very sensitive and that 'Maple Arrow' lies between them in regard to aluminium tolerance.

These results permit the conclusion that an *in*

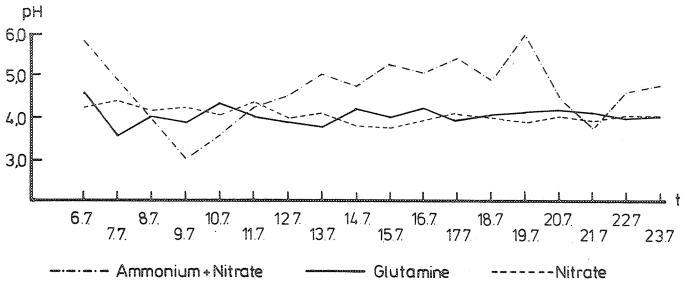

Fig. 4. The influence of different nitrogen compounds on the stability of the pH-value

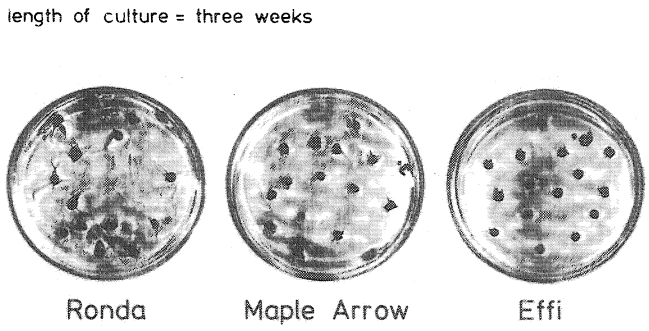

Fig. 5. The influence of 120 mg L Al^{3+} on the secondary root growth of three varieties

vitro screening method may also be used for aluminium, although other species must be tested for the applicability of the method to them. The three examples show that when these techniques are carefully integrated into an existing breeding programme, they can bring about an improvement in adapting genotypes for particular conditions and this could have tremendous implications for the future.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 345–353.
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PLSO PN174

Screening pasture plants for aluminum tolerance

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Key words: Al-tolerance, evaluation parameters, pasture plants, red clover cultivars, screening techniques

Abstract

Many investigators have reported that plant species and genotypes within species differ widely in tolerance to Al toxicity in acid soils. Considerable effort has been made to develop Al tolerant genotypes which would reduce the costs of conventional liming practices. However, forage plants have received much less attention than grain and other food crops. Forage species include large numbers of tropical and temperate season grasses and legumes differing widely in growth habits, seed size and initial growth rate. Such diverse characteristics require different techniques in screening these plants for tolerance to Al tocixity and other stress factors. The complex nature of acid soil "infertility" which involves interactions of Al (and Mn) with other mineral elements makes it difficult to simulate acid soil conditions in nutrient solutions. Yet the development of nutrient solution techniques is needed expedit the screening of large plant populations.

The objective of this presentation is to discuss screening techniques and parameters used to identify Al tolerant pasture plants. Data presented were derived from two experiments. The first experiment was conducted in pots of Monmouth soil treated with 0 or 750 ppm $CaCO_3$ (final pH 4,8 and 5,63, respectively). The second experiment involved 0 and 3 ppm Al in nutrient solution at an initial pH of 4,5. Seventeen red clover cultivars were used in each experiment. Top and root dry weight (TW-RW) root length (RL), tolerance index (TI = stress/unstresses %) and various combinations of these were compared in the two experiments. High correlation was observed between parameters within each experiment, particularly for TW and RW. Significant correlation was also found between soil and nutrient culture results for TW and RW parameters. Different scores were obtained for different plant characteristics used in both soil and nutrient solution studies; however some cultivars showed consistent classification indifferently of the parameter applied. Two parameters together (TW and RW) seemed most effective in screening plants within species. One can screen plants in nutrient solution with a single Al treatment and in soil at the lowest pH possible for the most tolerant cultivars to make reasonable growth. This approach makes it possible to reduce the number of treatments required or to increase the number of cultivars examined within a limited space and time.

Introduction

Aluminum (Al) toxicity limits plant growth on acid soils throughout the world (Foy, 1974; Olmos and Camargo, 1976; Silva, 1976). Many investigators have reported that plant species and genotypes within species differ greatly in tolerance to Al stress (Foy, 1976; Reid, 1976a), and that Al-tolerant plants may provide useful alternatives to the high costs of conventional liming practices (Foy, 1976; 1983; Howeler, 1987).

Breeders have expended great effort to identify Al-tolerant plant species and to increase Al-tolerance in plant genotypes (Reid, 1976a; Elgin, J.H. Jr. – personal communication). Pasture plants have received less attention than grain crops. This fact can probably be attributed to their lower economic importance and to the difficulties in screening procedures. Some of

Table 1. Examples of pasture plants screened for Al-tolerance

Common name	Scientific name	Reference
Turfgrass	*Poa pratensis*	Murray and Foy, 1978
Fescue	*Festuca rubra*	Murray and Foy, 1978
Tall fescue	*F. arundinacea*	Murray and Foy, 1978
Ryegrass	*Lolium multiflorum*	Nelson and Keisling, 1980
Leucaena	*Leucaena* spp.	Oakes and Foy, 1984
Leucaena	*L. leucocephala*	Tergas and Urrea, 1985
Elephant grass	*Pennisetum purpureum*	Tergas and Urrea, 1985
Non listed	*Axonopus scoparius*	Tergas and Urrea, 1985
Pegeon pea	*Cajanu cajans*	Tergas and Urrea, 1985
Limpograss	*Hemarthria altissima*	Foy and Oakes, 1984
Sorghum	*Sorghum bicolor*	Duncan *et al.*, 1983; Furlani *et al.*, 1982
Red clover	*Trifolium pratense*	Baligar *et al.*, 1985; 1987
White clover	*T. repens*	Baligar *et al.*, 1985; Caradus *et al.*, 1987
Alfafa	*Medicago sativa*	Baligar *et al.*, 1985
Bird food trefoil	*Lotus corniculatus*	Baligar *et al.*, 1985
Flatpea	*Lathyrus sylvestris*	Baligar *et al.*, 1985
Oat	*Avena sativa*	Bilski and Foy, 1987
Bluestens	*Botriochloa* spp.	Foy *et al.*, 1987
Centrosema	*Centrosema* sp.	Hutton, 1985

those pasture plants studied for Al-tolerance are presented in Table 1.

The difficulties of screening for Al-tolerance are increased because pasture and forage species include large numbers of tropical/subtropical of grasses and legumes species and genotypes within each species. These differ widely in growth cycles (annual, biennial or perennial), growth habit (erect or prostate) seed size (including hard seeds) and initial growth rate. This diversity of properties may require the use of different screening methods.

The objective of this paper is to discuss some screening techniques and parameters that can be used to identify Al-tolerant pasture plants as well as to suggest a new proposal of using two parameters together. This approach is based on data from two trials carried out by Foy and Nuernberg, 1985 (unpublished) in pots of soil treated with 0 or 750 ppm $CaCO_3$ and nutrient solution with 0 or 3 ppm Al. Seventeen red clover cultivars (*Trifolium pratense* L.) were grown in each trial.

Screening techniques

Numerous techniques have been used in screening for tolerance to Al stress using either nutrient solution or soil procedures (Foy, 1976; Howeler, 1987; Konzak *et al.*, 1976; Moore *et al.*, 1976; Reid, 1976b; Rhue and Grogan, 1976), particularly for non forage species. The same procedures have been used for some pasture plants screenings.

Subjects involved in using soil and nutrient solution (concentrations of nutrients, toxic elements, volume of solution and different types of soil) have been well discussed by Foy (1974, 1976) and Howeler (1987). These variables must be investigated when preliminary knowledge for a given species is insufficient. Preliminary tests are often necessary to determine adequate low and high soil levels pH and Al concentrations, respectively for soil and nutrient solution media that are best for subsequent screening proceeding (Foy, 1976).

Each screening procedure has advantages and disadvantages (Howeler, 1987). Nutrient solution and soil pot techniques are normally conducted under partially controlled conditions (growth room or greenhouse). These methods are generally more rapid and cheaper, and permit screening of larger numbers of entries with limited space and seed supplies than could be accomplished under field conditions. Hence, field testing can be confirmed to genotypes showing extreme tolerance differences in preliminary

tests (Bilski and Foy, 1987; Campbell and Lafever, 1976; Furlani *et al.*, 1982). Field tests must be conducted to obtain the final results of the material classified as Al-tolerant in controlled trials. Standard varieties previously known should be included in each test to adjust for slight changes in the experimental conditions (Foy, 1976; Howeler, 1987).

Nutrient solution technique is particularly recommended for plant breeders who can later transplant the best material to the field. Also this technique is considered the most suitable for plants that grow from seeds with small nutrient reserves and rapid initial growth (Howeler, 1987). Many pasture plants have these characteristics, especially legume forage species. Small seeds require more care in their management; hence forage screening require different equipment from that used for grain crops.

Nutrient solution is considered more precise because important variables like pH control, elements balance, *etc.*, can be better controlled (Moore *et al.*, 1976). Soil tests are less precise because Al tocixity is not the only limiting factor in acid soils, and soil changes other than available Al occur in limed pots or plots. Nevertheless, Foy (1976) suggests that screening work begin with natural soils (frequently subsoil) which have a pH value at 4.5 to 5.0 and low Mn content.

Many authors have found high correlations between Al-tolerance of grain crops in controlled conditions and those in field tests (Bilski and Foy, 1987, Campbell and Lafever, 1976; Foy, 1976; Howeler and Cadavid, 1976; Reid, 1976b). However, comparable research reports for pasture plants are rare or non-existent.

All of the factors previously presented make comparison of experimental figures difficult. Based on these problems Fageria *et al.* (1988) recommended standardizing experimental conditions as much as possible.

Plant variables for screening

The literature presents various plant variables for different screening procedures. The following are the main parameters used: grain yield (field trials); tops and roots weight (field, soil-pot and nutrient solution trials); top and root length (tap root, seminal root and branching, *etc.*) and Al-tolerance index (relative root or shoot weight or length at high and low Al in nutrient solution or at low pH and high pH in soil-pot trials). Within a given experiment this parameters are normally well correlated, although, some deviations occur. These deviations are attributed to genetic differences among cultivars or strains. This make clear that different results can be obtained when different variables are used separately. Thus, some desirable genotypes can inadvertently be eliminated.

The main plant variables for Al-tolerance screening will be discussed emphasizing pasture plants using the proposed trials.

Top and root weight

Absolute top weight (TW) and root weight (RW) from both no Al-stress and Al-stress conditions (soil or nutrient solution) are obtained for identifying yield potential of each tested genotype. Top weight is particularly important when pasture plant genotypes are being studied because it represents the final product used as feed. This parameter (TW) is more easily obtained in all growth media. Many authors, on the other hand, have used root parameters because Al is characteristically more harmful to roots than to tops (Foy, 1976; Oakes and Foy, 1984). However, the assessment of RW is more difficult in soil trials and in the field it is practically impossible.

The RW can be widely affected by root manipulation during the cleaning procedure either by the losses of fresh material or by the remaining soil particles among the roots.

Actual RW and TW are not normally used directly as attributes in identifying Al-tolerant or susceptible genotypes. Perhaps, statistical analysis could be adopted in these cases using particularly data from the stress condition; however data in Table 2 for red clover as well as that from many other papers, show clearly the difficulty in obtaining well defined groups of genotypes both in soil and nutrient solutions trials by applying a statistical test.

Significant correlation was observed between TW and RW in soil experiments ($r = 0.81^{**}$) and in nutrient solution trials ($r = 0.93^{**}$). Problems in cleaning roots discussed previously, may have

Table 2. Actual top and root dry weights of 17 red clover cultivars grown on acid Monmouth soil at two lime levels and and in nutrient solution at two aluminum levels

	Soil-pot experiment (mg/pot)				Nutrient solution trial (mg/pl[a])			
	Unlimed (1)		Limed		No aluminum		With aluminum	
Cultivar	TW[b]	RW[c]	TW	RW	TW	RW	TW	RW
Arlington	478 a[d]	263 ab	672	465	19.9	5.5	14.0 ab	5.0 a
Redland II	465 ab	300 a	585	365	16.9	4.6	11.8 abcde	4.1 abc
Reddy RC	443 ab	239 abc	584	392	12.8	4.1	11.0 abcde	3.9 abcd
YKYC	436 abc	241 abc	508	292	16.6	4.4	11.4 abcd	3.9 abcd
Tristan	434 abc	236 abc	531	326	15.7	4.7	12.7 abc	4.2 abc
Florie	426 abc	211 abc	581	343	14.8	4.6	4.6 a	4.6 ab
X78045	420 abc	196 bc	584	395	20.9	5.4	9.1 abc	2.6 d
Redman	419 abc	215 abc	536	351	16.4	5.1	10.8 bcde	3.5 abcd
Lakeland	415 abcd	243 abc	558	380	19.1	5.7	13.3 ab	5.0 a
Florex	405 abcd	190 bc	548	343	12.0	2.9	10.4 bcde	3.6 abcd
Prosper I	394 bcd	200 bc	504	288	13.7	3.9	13.3 ab	4.2 abc
78122	381 bcd	228 abc	574	353	13.1	3.3	12.2 abcd	4.2 abc
Chesapeake	375 cde	172 bc	565	288	11.4	3.3	11.6 abcd	4.0 abcd
X78044	375 cde	251 abc	566	346	11.4	3.7	11.4 cde	3.9 abcd
Kenland	335 def	171 bc	470	291	12.0	4.0	7.5 e	2.9 cd
ISI-84-KM	302 ef	152 c	501	228	10.5	3.2	8.9 de	3.3 bcd
Kenstar	274 f	158 c	421	407	9.7	3.0	8.9 de	2.9 cd

[a] Initial soil pH = 4.8; nutrient solution pH = 4.5.
[b] TW = top dry matter weight.
[c] RW = root dry matter weight.
[d] Means within a column followed by the same letter don't differ significantly at the 5% level by Duncan's Test.

reduce correlations in the soil. Root and top weight could be adopted, in these trials, considering the high correlation between those variables. However, different screening results can be observed in Table 2 if only one of these parameters is used.

Correlation analysis under stress conditions confirmed the relationship between soil and nutrient solution for RW ($r = 0.62^{**}$) and TW ($r = 0.57^{**}$). Deviations between results of both trials can be explained by the fact that nutrient solution studies may not simulate the mineral environment of the soil (Bennett *et al.*, 1986).

Root and top length

Roots are the first plant organs affected by Al-tocixity; injury is manifested through reduced elongation and increased thickness (Baligar *et al.*, 1987; Foy, 1974). Hence, root length (RL) might be a useful plant parameter for Al-tolerance screening.

Root length measurements can be obtained easily in nutrient solution studies whereas in field trials it is practically impossible. In soil-pot trials similar problems reported for root weight are also observed here.

Root length is more commonly obtained by measuring seminal roots in short-term trials. However, seedling root length can be highly affected by mineral seed reserves, genetic variability and lack of germination uniformity (Furlani *et al.*, 1982; Moore *et al.*, 1976; Nelson and Keisling, 1980), masking the Al-toxicity effects. Such problems can be reduced by selecting pregerminated seedling for testing.

Fageria *et al.* (1987), suggested longer term trials, at least 3 or 4 weeks, when Al-toxicity can be evaluated in plant tops. In this situation direct root measurement is less precise mainly for grass species which have large number of adventitious roots. Thus, a method capable of evaluating total length of all roots of plants grown in experimental unit as such that proposed by Tennant (1975) could be more precise. Unfortunately these methods can be quite time consuming.

Better results can be obtained through root measurement on individual plants (Moore *et al.*,

1976). For this evaluation procedure, the number of genotypes need not be so high for the trial, neither the number of plants in each experimental unit. Nevertheless, when pasture genotypes grow from seeds are screened, no less than 30 seedlings of the same genotypes per treatment should be used because of their high genetic variability (Elgin, Jr. personal information).

Absolute RL is not directly used for screening procedure. If it is adopted, statistical tests can be used to identify Al-tolerance differences between genotypes. However, data from 17 red clover cultivars grown in nutrient solution (Table 3) show that this procedure presents difficulties in obtaining clear separation Al-tolerant or Al-susceptible red clover cultivars as also observed for top and root weight.

Red clover genotypes grown in nutrient solution showed low but significant correlation between tap root length and RW ($r = 0.52^*$) and a non significant correlation between tap root length and TW ($r = 0.40$ NS) under Al-stress conditions. Deviations of these correlation were probably caused by differential behavior of some cultivars in presence of Al, reducing root elongation and increasing root thickness with only little reduction in root weight. Similar results were observed by Mesday and Balkema-Boonstra (1984) and Dambroth and El Bassan (1982).

Table 3. Actual average root length of 17 red clover cultivars grown on nutrient solution with Al and no Al

	Root length (mm)	
Cultivar	No aluminum	with aluminum
Arlington	130	97 abcd
Redland II	117	80 d
Reddy RC	110	93 abcd
YKYC	123	100 abc
Tristan	123	103 ab
Florie	143	97 abcd
X78045	130	83 d
Redman	130	93 abcd
Lakeland	150	110 a
Florex	133	100 abc
Prosper I	120	83 cd
78122	123	83 cd
Chesapeake	117	93 abcd
X78044	123	100 abc
Kenland	127	87 bcd
ISI-84-KM	117	90 bcd
Kenstar	110	83 cd

Few papers present plant height in screening studies, but this could be a useful variable in longer term trials when tops present Al-tocixity effects. The measurement is easily obtained in all types of trial, field or controlled conditions.

A significant correlation was observed between plant height and top weight for *Medicago sativa* plants grown in acid soil or nutrient solution (Nuernberg, *et al.* unpublished data) and for *Lotus corniculatus*, *Trifolium pratense*, *T. repens*, *Ornithopus* sp., *Festuca arundinacea*, *F. rubra* and *Lolium multiflorum* plants grown in soil-pot trials (Nuernberg and Plandolfo, unpublished data). In the same trial, however, this correlation was not observed in plants of *Agrostis tenuis* and Holcus lanatus. This fact means that knowledge is required concerning this parameter, before it can be used to screen a given species.

For plant breeders plant height and root length are particularly interesting because these measurements can be made while measuring the best material and transplanting it to the field, particularly when the seed supply is limited.

Relative growth (Al-tolerance index – TI)

Literature reporting the screening for Al tolerance in plants shows that relative growth, often called Al-tolerance index (TI), is the most used parameter to separate genotypes. Plant growth features (root weight, top weight, *etc*) are expressed as high stress/low stress conditions in percentages. Thus, two lime levels or Al concentration are necessary in trials.

TI is necessary when comparing different species or genotypes within species that differ widely in growth habits (Foy, 1976) or when comparing results from trials carried out in different places or years where the absolute values would differ (Campbell and Lafever, 1976).

Many workers apply statistical analysis on TI values to separate Al-tolerant or Al-susceptible genotypes. However, statistical tests are often not sufficiently clear to separate those different groups according to their Al-tolerance.

Another possibility for genotype separation is through the establishment of classes of TI in which genotypes are grouped as Al-tolerant, sus-

Table 4. Relative top and root yield (%) and root length (%) of red clover cultivars grown in acid Monmouth soil at two lime levels and in nutrient solution at two aluminum concentrations

	Soil trial[a]		Nutrient solution trial		
Cultivar	TW[b]	RW[c]	TW	RW	RL[d]
Arlington	71	56	70	91	75
Redland II	79	82	70	89	68
Reddy RC	76	61	86	95	85
YKYC	86	83	69	89	81
Tristan	73	62	99	100	84
Florie	73	62	99	100	68
X 78045	72	50	44	44	64
Redman	78	61	66	69	72
Lakeland	74	64	70	88	73
Florex	74	55	87	129	75
Prosper I	78	69	97	108	69
78122	66	79	93	127	67
Chesapeake	66	60	102	121	79
X 78044	66	73	100	105	81
Kendland	71	59	60	73	69
ISI-84-KM	60	67	85	103	77
Kenstar	65	39	92	97	75

[a] Soil: unlimed/limed %; Nutrient solution: with Al/no Al%.
[b] TW = top dry weight.
[c] RW = Root dry weight.
[d] RL = tap root length.

ceptible or intermediate. Using this criteria for red clover TI from soil-pot and nutrient solution experiments (Table 4), different genotypes are classified depending on the medium and relative growth parameter used. The diversity of screening results presented in Table 4 can be caused by factors other than Al-toxicity in each medium, according to what was discussed before about absolute values of absolute plant parameters.

According to Howeler (1987), TI tends to separate all varieties highly affected by Al-toxicity, it also tends to include the truly Al-tolerant germplasm and those Al-susceptible varieties having a low yield potential without stress. Thus, these varieties, selected as having the highest TI often have low yield under both high and low stress. This fact, for example, was also evidenced in Kenland and Arlington red clover cultivars. Both presented the same TI value for TW parameter (Table 4) but Kenland produced only 70% as much top weight as Arlington on the unlimed soil (Table 2). Similar results were observed by Baligar *et al.* (1987) working with 23 red clover cultivars in nutrient solution. Based on several experiments conducted in CIAT (Centro Interamericano de Agricultura Tropical), Howeler (1987) presented different alternatives that could be used in order to solve this kind of problem.

Screening by root and top yield combination

Red clover screening for Al-tolerance by absolute or relative growth of roots and tops discussed previously, showed that each parameter classified genotypes differently according to yield potential at high Al-stress. Only a few varieties showed consistent behavior under high or low Al-stress independently of applied parameter or growth medium. This fact may cause doubt in the choice parameters adopted for that purpose. Perhaps a combination of two plant parameters, specially those involving both tops and roots, could present more consistent results.

A method that could be used for trials under controlled conditions (pots-soil and nutrient solution) is presented using results with 17 red clover cultivars to support the discussion. For this proposal only the high Al-stress level is necessary. For soil-pot trials the original soil pH

(not limed) can be used and an unstressed condition can be imposed by suitable liming which permits growth of the most Al-tolerant genotype (Foy, 1976). For nutrient solution the Al-stress condition must be created by addition of Al at a pH low enough to maintain its solubility. For both media preliminary studies are necessary to determine suitable stress conditions for screening a given species.

Adapting suggestions presented by Howeler (1987) and Fageria *et al.* (1988), we used values of root parameter on the X axis and actual values of top parameter on Y axis. The graph is divided into four fields, using lines of average X and Y values or average of X and Y plus 0.5, 1.0 or more standard deviations (SD), according to the rigor of the study. Thus, four well defined categories of genotypes are obtained are according to their response to high Al-stress. Members of each category can be classified according to their capacities to yield roots and tops under high Al-stress. The categories are: 1. Al-tolerant – cultivars having good yields of both tops and roots; 2. Al-susceptible – cultivars having poor yields of both tops and roots; 3. Intermediate (a) – cultivars having good top yields but poor root yields; 4. Intermediate (b) – cultivars having poor top yields but good root yields.

For red clover cultivars, absolute values of TW and RW from the soil-pot trial are plotted in Figure 1, and TW and RW (or RL) from the nutrient solution experiment are plotted in Figure 2. Cultivars classified according to this procedure are shown in Table 5 for soil and nutrient solution media.

The results achieved show that well defined groups of cultivars can be distinguished. This method seems advantageous because all varieties

Table 5. Aluminum tolerance of red clover cultivars based on root and top dry weights under Al stress

Soil-pot experiment	Nutrient solution experiment	
Top and root weight	Top and root weight	Top weight and root length
Al-tolerant (high root and top yield)		
Redland II	Arlington	Lakeland
Arlington	Lakeland	Tristan
Reddy RC	Florie	Florie
YKYC	Prosper I	Arlington
Lakeland	Tristan	YKYC
Tristan	78122	X78044
–	Redland II	Chesapeake
–	Chesapeake	Reddy RC
–	YKYC	–
Intermediate 'a' (low root yield height top yield)		
Redman	X78044	Prosper I
Florie	–	78122
X78045	–	Redland II
Florex	–	–
Intermediate 'b' (high root and low top yield)		
X78044	Reddy RC	Florex
78122	–	Redman
Al-susceptible (low root and top yield)		
Prosper I	Florex	ISI-84-KM
Chesapeake	Redman	Kenland
Kenland	ISI-84-KM	X78045
ISI-84-KM	Kenstar	Kenstar
Kenstar	X78045	–
–	Kenland	–

can be easily observed at the same time using the most important parameters – roots and tops together.

Another advantage of this approach is that ony one experimental stress level is necessary in the trials consequently the number of cultivars can be increasing within given space and time, or the screening work can be reduced to the half and reduce costs.

It is necessary to keep in mind that previous knowledge about the Al stress level for a given forage species is required. Another important necessity is that standard indicator cultivars (tolerant and susceptible) is grown simultaneously as check (Fageria *et al.*, 1987; Foy, 1976).

Conclusion

Several grass and legume species and genotypes within species have been identified which present acceptable growth under Al-stress conditions. Selection of Al-tolerant plants can reduce the lime requirement for acid soils and various screening techniques and plant parameters that can be useful for this selection have been reported. Relative top or root growth (tolerance index) is probably the most widely used parameter, and absolute values have had little use in screening procedures. In the same trial, different results might be obtained by using different top or root parameters. Aluminium is characteristically more harmful to roots than tops; thus, root growth parameters should be used in plant screening. However, top dry matter yield potential is an important feature for pasture plants and must also be considered. The combination top and root parameters is believed to give more consistent results in pasture screening plants. However, results obtained in this way must be later confirmed in field tests, with the best materials.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 355–360.
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PLSO PN 175

Screening soybean for aluminium tolerance and adaptation to acid soils

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Key words: aluminium, genotypic differences, roots, soil acidity, soybean

Abstract

Inhibition of root elongation was the most sensitive response of soybean plants to Al in solution culture. Primary-root elongation was confined to the apical 10-mm root tip. Initial response of root elongation to Al seemed to be due to inhibition of cell elongation rather than cell division. Negative correlations existed between Al concentrations of root tips and genotypic root length at low and high Al supply. Using the product of the primary-root length and the longest secondary-root length after 4 days of Al treatment ($74\,\mu mol\,L^{-1}$) relative to the control without Al as parameter, 938 soybean genotypes from the world soybean collection were screened for Al tolerance. Large genetic variability could be found. Elsewhere reported differences in Al tolerance of selected soybean genotypes could be confirmed. However, no relationship existed between genotypic Al tolerance in solution culture and adaptation to an acid soil in Southeast Nigeria reported by IITA (Annual Report, 1981). Therefore a sand-culture screening procedure was established. Compared to solution culture, a 10 times higher Al concentration was necessary to inhibit root growth to a comparable degree. There was a significant but rather weak correlation ($r = 0.43$) between the responses to Al of 31 genotypes in solution and sand culture. Genotypic response of root growth to Al in sand compared to solution culture was more closely, but still not significantly, related to growth of the genotypes in acid soil under field conditions in Nigeria. This indicates, that inhibition of root growth by Al was not primarily responsible for growth reduction due to soil acidity under those field conditions.

Introduction

In acid mineral soils, Al toxicity is the most important soil factor limiting plant growth. This is especially true for soybean, which may be classified as less well adapted to acid soils with high Al supply compared to other plant species (Munns and Fox, 1977). However, large differences in Al tolerance between soybean genotypes could be identified (Devine *et al.*, 1979; Sapra *et al.*, 1982), encouraging the breeding of cultivars for adaptation to acid soils (Hanson and Kamprath, 1979).

Breeding progress very much depends upon the availability of quick screening techniques allowing the screening of large numbers of genotypes. Screening techniques for Al tolerance under controlled conditions have been described by several authors (Foy *et al.*, 1974; Furlani and Clark, 1981; Reid *et al.* 1971). They are primarily based on the exposure of seedlings to Al in solution or soil culture and measurement of root growth inhibition by Al. However, information about relationships between ranking of genotypes for Al tolerance in the seedling stage under controlled conditions and for adaptation to acid soils under field conditions are scarce.

The objectives of these studies were (1) to identify the most sensitive response of soybean to Al, (2) to evaluate the Al tolerance of a larger number of genotypes of the world soybean collection, and (3) to relate Al tolerance under controlled conditions to results from field experiments on acid soil in Southeast Nigeria reported by IITA (1981).

Material and methods

Seeds of the soybean genotypes (*Glycine max* L.)

were supplied by International Institute of Tropical Agriculture, Ibadan, Nigeria, USDA Soybean Germplasm Collection Urbana Illinois, USA, and Kleinwanzlebener Saatzucht AG, D-3352 Einbeck.

Growing conditions of plants

Plants were grown in a growth chamber of 30°/25°C day/night temperatures, 16 h daylength, 150 W m^{-2} light intensity (Osram HQI-T 200 W/D lamps), and 70% relative humidity. Seeds were germinated for 2 days between filter paper soaked with 1 m*M* $CaSO_4$. Subsequently seedlings were transferred to 22 L plastic vessels containing nutrient solution with the following composition (μM): KNO_3, 750; $Mg(NO_3)_2$, 325; KH_2PO_4, 10; $CaSO_4$, 250; H_3BO_3, 8; $CuSO_4$, 0.2; $ZnSO_4$, 0.2; $MnSO_4$, 0.2; $(NH_4)_6Mo_7O_{24}$, 0.2; FeEDDHA, 20. Al was added as $AlCl_3$. The pH of the nutrient solution was adjusted to 4.2 and kept constant with 0.01 *M* HCl or KOH by automatic titration.

Mass screening in solution culture

In contrast to all other experiments, seeds were germinated in a peat/sand mixture for 2 days at 30°C, then carefully washed in distilled water and transferred to two 250 L tanks (+, − Al) containing the above described nutrient solution. KH_2PO_4 was supplied at 64 μM. Al was added as $Al_2(SO_4)_3$. The pH was adjusted daily to 4.2 using 0.1 *M* H_2SO_4. 130 genotypes with 3 single plant replicates per Al treatment could be screened at the same time. The experiment was repeated 9 times with different genotypes using 14 genotypes as internal standards. After 4 days plants were harvested and lengths of the primary roots and the longest secondary roots measured with a ruler.

Screening in sand culture

After germination the seedlings were transferred to 3 L plastic pots filled with quartz sand (grain diameter appr. 1 mm). The sand was leached two times per day with 336 mL kg^{-1} (corresponding to 2 × water capacity) of fresh nutrient solution of the same composition as in solution culture (see above). After 4 days, roots were washed free of sand and fresh weight determined.

Determination of root-elongation and cell-division rate

At the beginning of the Al treatment roots were marked with a black marking pen 2 cm or at 2 mm distances from the apex. Distances from the marks to the apex and between the marks were measured (+, − 0.05 mm) after different periods of Al treatment. Marking of the roots and measurements did not affect root elongation (preliminary experiments). Parallel to the measurements of root elongation, two plants per replicate (5) were harvested. 1 mm tips of the primary roots were cut off and immediately fixed in ethanol/acetic acid (3:1) for 24 h at 4°C. Root tips were then stained with carmine-acetic acid for 5 days at room temperature. For the determination of the rate of cell division, root tips were carefully squashed, numbers of mitotic nuclei counted under a microscope (Zeiss), and expressed as percent of total number of meristematic cells in the same microscopic field (about 100 cells).

Results

Root elongation was much more sensitive to Al supply than root and shoot dry matter production (Fig. 1). At the relatively high Al supply primary and secondary-root growth were equally inhibited by Al. At lower Al supply, however, secondary roots were more sensitive (not shown). As a consequence of reduced root elongation, branching density was slightly increased and specific root dry weight greatly increased, indicating thicker roots. The main elongation zone of the primary root was the zone 4–8 mm from the apex. This may be concluded from the inhibition of root elongation by Al of different root tip sections (Fig. 2).

Inhibition of root elongation could be measured as early as 1 h after beginning of the Al treatment (Fig. 3). Owing to the high Al supply during this short term experiment, root elongation nearly completely stopped after 8 h of Al treatment. The numbers of mitotic nuclei were later and less severely affected by Al, indicating that inhibition of

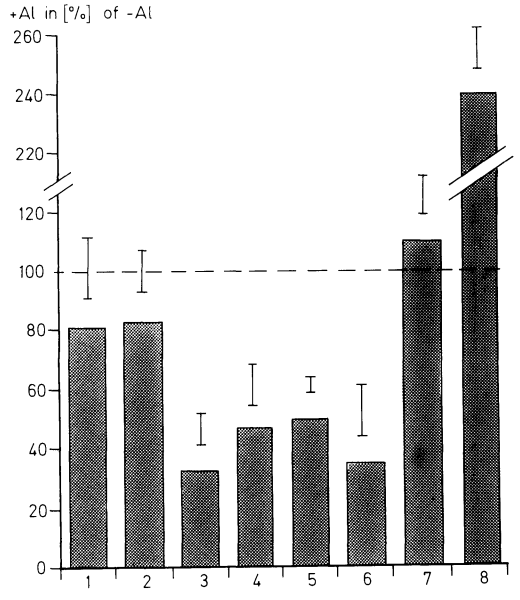

Fig. 1. Effect of aluminium supply (74 μM) in solution culture on shoot and root growth of soybean ('Gieso'). Duration of Al treatment 4 days. *Bars* indicate LSD 0.05 (Al).
1 Shoot dry weight.
2 Root dry weight.
3 Total root length.
4 Primary-root length.
5 Mean secondary-root length.
6 Distance between primary-root apex and the first, most recently emerged secondary root.
7 Number of secondary roots per cm of secondary-root zone of the primary root.
8 Root dry-weight/root-length ratio.

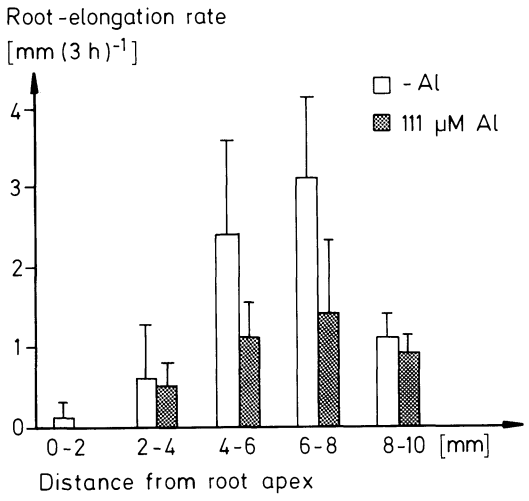

Fig. 2. Effect of aluminium supply on elongation rate of sections of primary roots of intact soybean seedlings ('Maple Arrow'). Duration of Al treatment 3 h. Mean of 8 replicates. *Bars* indicate SD.

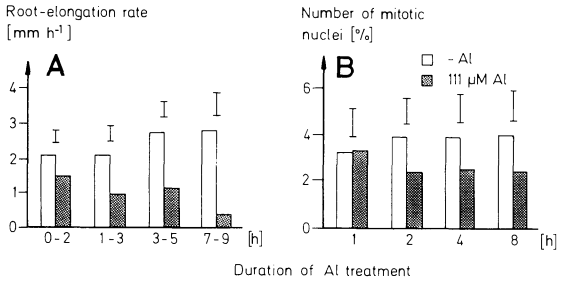

Fig. 3. Effect of the duration of the aluminium supply on elongation rate (**A**) and on cell division rate (**B**) (number of mitotic nuclei per 100 cells) in root tips of primary roots of soybean seedlings ('Maple Arrow'). *Bars* indicate LSD 0.05 (Al).

root elongation was due to inhibition of cell elongation rather than cell division.

Negative correlations existed between Al concentrations of root tips and absolute root length of 7 genotypes at low (not significant) and high Al supply (Fig. 4). This suggests that differences in response of root elongation to Al between genotypes were due to differences in Al uptake into the most Al-sensitive root tissues.

Using the product of the primary-root and the longest secondary-root length after 4 days of Al treatment as parameter, which was highly significantly correlated to total root length (not shown), 938 soybean genotypes from the world soybean collection were screened for Al tolerance (Fig. 5). Independent of whether absolute or relative root length was used as parameter, the majority of the genotypes were classified as Al-sensitive. A few genotypes with outstanding Al tolerance could be identified. Ranking of the genotypes for Al tolerance agreed fairly well with results reported in the literature (Table 1).

However, no relationship existed between genotypic Al tolerance in solution culture and genotypic adaptation to an acid soil with high Al supply in Southeast Nigeria reported by IITA (1981) and recent unpublished results (not shown). This could be due to mechanical impedance to root growth modifying Al tolerance. Therefore a sand-culture screening-procedure was established. Compared to solution culture, in sand culture a higher Al concentration (at least 10 times) in the nutrient solution was necessary to inhibit root elongation to

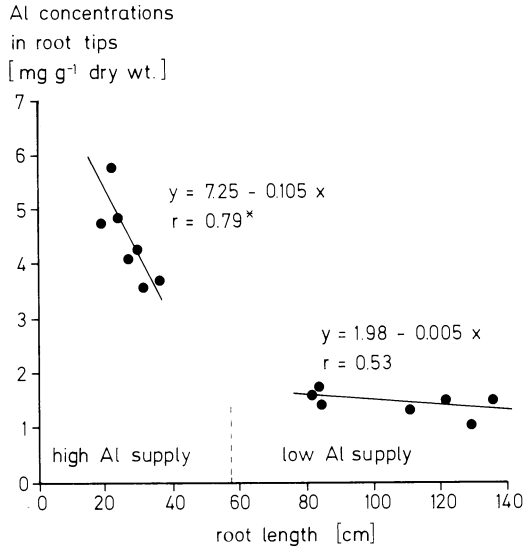

Fig. 4. Relationship between root length and aluminium concentrations in root tips of 7 soybean genotypes at low (18.5 μM) and high (70.1) Al supply. Duration of Al treatment 4 days.

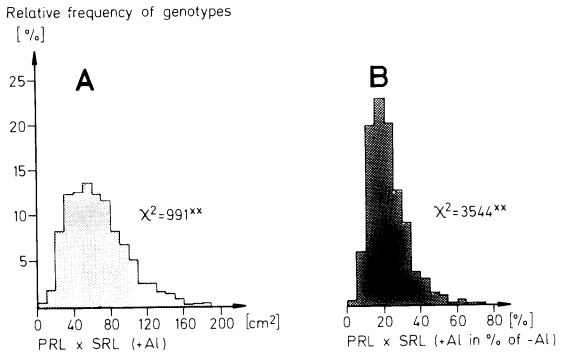

Fig. 5. Frequency distribution of 938 soybean genotypes as to Al tolerance in solution culture based on
A: absolute root length at 74 μM Al supply.
B: relative root length at 74 μM Al supply. Control (−Al) = 100%.
Duration of Al treatment 4 days.
PRL = Primary-root length.
SRL = Length of longest secondary root.

a comparable degree (Table 2). Response of root growth of 31 genotypes to Al solution and sand culture was only weakly correlated (Fig. 6) which confirmed the above made assumption. Root fresh weight was taken as parameter for root growth in sand culture, because it was highly correlated to total root length and much easier to determine

Table 1. Aluminium tolerance of selected soybean genotypes based on absolute and relative lengths of primary roots (PRL) and secondary roots (SRL) after 4 days of Al treatment (74 μM) in comparison to classification of the genotypes for Al tolerance in previous studies by different authors

Genotype	PRL × SRL + Al absol. (cm^2)	PRL × SRL + Al in (%) of − Al	Classification for Al tolerance compared to other authors
PI 417021	185.1	99.9	most tolerant (this study)
Norchief	108.1	37.8	tol. (Arminger *et al.*, 1968)
Hawkeye	104.2	35.5	tol. (Arminger *et al.*, 1968)
Richland	98.5	31.4	tol. (Konzac *et al.*, 1977)
Mandarin	78.9	22.2	tol. (Arminger *et al.*, 1968)
Mukden	77.8	24.9	tol. (Foy *et al.*, 1969, Sopra *et al.*, 1982)
Perry	76.4	28.6	tol. (Foy *et al.*, 1969, Sopra *et al.*, 1982)
Flambeau	57.9	20.9	sensitive (Konzac *et al.*, 1977)
Manchu	57.3	13.6	sensitive (Konzac *et al.*, 1977)
Capital	47.0	17.6	sensitive (Arminger *et al.*, 1968)
Kent	42.1	12.4	more tol. than Bragg (Sartain and Kamprath, 1978)
Forrest	39.5	16.4	more tol. than Bragg (Brown and Jones, 1977)
Amsoy	38.9	11.4	more tol. than Bragg = Kent (Sartain and Kamprath, 1978)
Merit	33.4	13.1	sensitive (Arminger *et al.*, 1968)
Clay	31.4	8.9	
Lindarin	28.2	10.8	sensitive (Konzac *et al.*, 1977, Arminger *et al.*, 1968)
Swift	27.4	8.2	
Bragg	23.3	8.6	sensitive, more sensitive than Kent and Forrest (Sartain and Kamprath, 1978, Brown and Jones, 1977)
Chief	22.3	8.6	sensitive (Foy *et al.*, 1969, Sapra *et al.*, 1982)
PI 423811	7.9	2.4	most sensitive (this study

Table 2. Effect of aluminium supply on total root length of soybean seedlings (mean of "Ronda" and "Maple Arrow" in solution and soil culture. Duration of Al treatment 4 days. Different letters indicate significant differences between substrates and Al supplies at $P = 0.95$.

Substrate	Al supply (μM)	Total root length/plant		
		−Al (cm)	+Al (cm)	+Al in (%) of −Al
Solution	74	189a	39d	21
Sand	741	114b	50c	44

(preliminary results). Ranking of the genotypes for Al tolerance in sand culture was more closely but still not significantly correlated to ranking for adaptation to acid soil under field conditions (not shown).

Discussion

Inhibition of root elongation was the most sensitive response of soybean to Al (Fig. 1). Results of Clarkson (1965), Morimura *et al.* (1978), and Horst *et al.* (1983) with different plant species indicate, that depression of cell-division rate is primarily responsible for Al injury to roots. Our results with soybean, however, suggest, that cell elongation is even more sensitive to Al (Fig. 3). This discrepancy might be due to the following reasons: (1) Measurement of root elongation was not sensitive enough in the previous studies, (2) Al concentrations of the nutrient solutions were excessively high in the previous studies, (3) Plant species react differently. It is especially attractive to speculate that in Al-sensitive plant species, the toxic effect of Al is mainly confined to the apoplasm, but in Al-tolerant species it is expressed in the symplasm due to complexation in the apoplasm and thus better permeability of the plasma membrane for Al.

Al tolerance of soybean genotypes was much higher in sand compared to solution culture (Table 2). This might be explained by (1) slower Al movement to the roots (diffusion in sand, convection in solution culture), (2) build up of a pH gradient at the root surface leading to precipitation of Al (Taylor and Foy, 1985), (3) enhanced release of root exudates (Barber and Gunn, 1974) which bind/inactivate Al (Horst *et al.*, 1982). There is good evidence that increase of pH in the rhizosphere is of minor importance, since NH_4^+

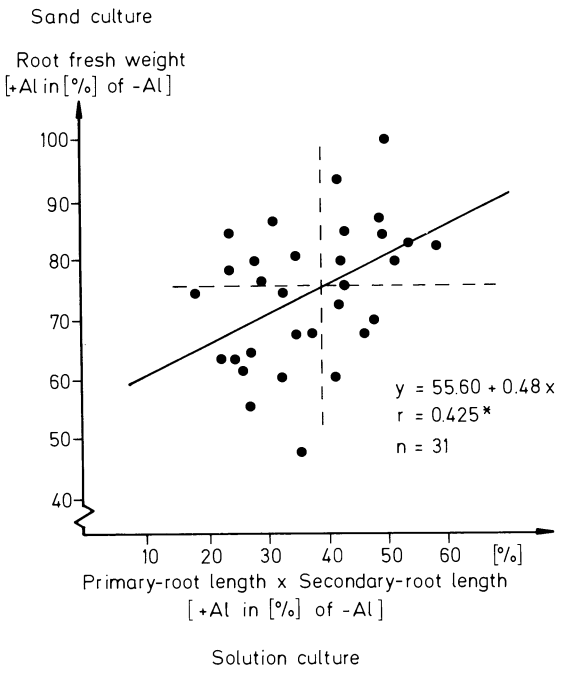

Fig. 6. Relationship between the responses in root growth of 31 soybean genotypes to aluminium in solution (74 μM Al) and sand culture (741 μM). Duration of Al treatment 4 days.

nutrition (inducing pH decrease) did not reduce but rather improved Al tolerance in sand culture (Klotz and Horst, 1988).

Screening for Al tolerance revealed large differences in Al tolerance between soybean genotypes (Fig. 5), which confirmed results of Devine *et al.* (1979) and Sapra *et al.* (1982). Also, large differences between genotypes in adaptation to acid soils were shown (IITA, 1981; Nicholaides *et al.*, 1983). However, no clear relationship existed between Al tolerance in the seedling stage in this study and adaptation to an acid soil with high Al supply under field conditions (IITA, 1981, unpublished results). Such lack of correlation between pot culture and field experiments seems to be characteristic of legumes. Good correlations have been reported for grasses (Lafever *et al.*, 1977; Reid *et al.*, 1969). Reasons for the lack of correlations could be, that

(1) seedlings and adult plants differ in Al tolerance. Devine *et al.* (1979) found a positive correlation between seed size and Al tolerance in solution culture, which was confirmed by our results (not shown).

(2) nutritional and soil physical factors which may differ under controlled and field conditions modify Al tolerance. The rather weak correlation between responses to Al of soybean genotypes in sand and solution culture (Fig. 6) emphasizes the importance of mechanical resistance for Al tolerance.

(3) Al (soil acidity) interacts with the N_2-fixing rhizobium/legume symbiosis (Hohenberg and Munns, 1984). This was probably of minor importance for the discussed comparison, because plants were independent of N_2 fixation due to application of fertilizer N in the field experiments (IITA, 1981).

(4) inhibition of root growth by Al was not primarily responsible for growth reduction in the acid soil under field conditions. Other factors could be Ca, Mg, P deficiencies induced by high H^+ and Al supply.

The results clearly show that improvement of adaptation of soybean to acid soils through selection based on quick screening techniques requires a better understanding of the physiological reasons for genotypic Al tolerance, of the factors that modify Al tolerance, and of those factors which primarily limit soybean growth in an acid soil of a specific site.

Acknowledgements

The work was part of a cooperative program with the International Institute of Tropical Agriculture, Ibadan, Nigeria. It was financially supported by the Commission of the European Communities within the research program 'Science and Technology for Development'.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 361–369.
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PLSO PN 176

Selection parameters for assessing the tolerance of wheat to high concentrations of boron

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Key words: boron, screening, tolerance, toxicity, wheat

Abstract

Three wheat genotypes known to differ in response to high concentrations of boron in the field were grown under controlled glasshouse conditions in soil containing 0, 20 and 60 mg kg^{-1} applied boron. Plant growth was assessed both destructively and non-destructively at regular intervals to identify the responses to high concentrations of boron that best discriminated between the tolerant and the sensitive genotypes, and to determine the optimal growth stage at which to rate the genotypes.

The concentrations of boron in whole shoots, in pots, at the first node and boot stages and in straw at maturity reflected the responses of the three genotypes in the field. Halberd had the lowest tissue boron concentrations, while (W1*MMC)/W1/10 had the highest, but the relative difference in boron concentrations was greater at the early harvests than in the straw at maturity. In contrast to field results, the concentration of boron in grain was not an indicator of the concentration in shoots for the pot experiment.

The ranking of the three genotypes for total dry matter production in pots was the same as ranking for tolerance in the field and was consistent for plants at all growth stages. Likewise, the height of plants four weeks after sowing was an indicator of vigour at the high boron treatment.

The rate of development was reduced at the high boron treatment and development of the sensitive genotype was delayed more than the tolerant genotypes. Tillering was more sensitive to high concentrations of boron than development of the main shoot. The severity of symptoms of boron toxicity increased with increasing boron supply and for successively older leaves and the ranking of genotypes was consistent with that in the field.

It is possible to screen for boron tolerance in the field using both destructive and non-destructive tests on plants grown in pots in the glasshouse, but these tests need to be conducted prior to anthesis as there is better discrimination between genotypes at that time.

Introduction

Potentially toxic concentrations of boron have recently been found in sub-soils throughout cereal growing districts of South Australia (Cartwright *et al.*, 1984; 1986), and high concentrations of boron have been measured in the shoots and grain of both wheat and barley plants growing in these soils (Cartwright *et al.*, 1984; 1986; 1987). Significant yield reductions have been attributed to boron toxicity (Cartwright *et al.*, 1984; Rathjen *et al.*, 1987) and it is believed that yield reductions due to boron toxicity may be occurring over large areas of the cereal belt of South Australia. There are no economically feasible means of alleviating the soil condition, however extremely diverse genetic variation in the response of wheat to high concentrations of boron has been identified (Moody *et al.*, 1988; Rathjen *et al.*, 1987). The cultivation of varieties able to tolerate high concentrations of boron would minimise the yield reductions due to boron toxicity.

In previous studies we have compared the rela-

tive tolerances of genotypes at maturity (*e.g.* Paull *et al.*, 1988), however, scoring at an earlier stage would lead to more efficient screening in a breeding program. Ideally, the response to boron stress at the time of selection should predict the response to be expected when the same variety is grown to maturity in the field.

When rating varieties of an inbreeding species, the screening system may be destructive and based on a number of plants, but when screening a segregating generation the selected plants need to be grown to maturity so measurements should be non-destructive and applicable to single plants. This study describes the responses to boron, at various stages of development, of three wheat genotypes having different tolerances of boron. Non-destructive measurements and observations were compared to tolerance ratings based on measurements of plant production and concentrations of boron in whole shoots. The selected genotypes were also grown under naturally occurring high boron conditions in the field to confirm their previous responses. The applicability of selected parameters to a wider range of genotypes is also examined.

Materials and method

Genotypes

Three wheat (*Triticum aestivum* L.) genotypes reported to differ in tolerance of high concentrations of boron were used. The genotypes were Halberd (moderately tolerant), Warigal (moderately sensitive) and (W1*MMC)/W1/10 (sensitive). Their responses to boron are described by Cartwright *et al.* (1987) and Paull *et al.* (1988). Halberd and Warigal are among the most widely grown cultivars in South Australia, while (W1*MMC)/W1/10 is an advanced breeding line from the Waite Institute wheat breeding program and has been selected for high yielding ability under 'normal' boron conditions. The pedigrees of the three genotypes are—Halberd: ((Scimitar* Kenya C6042)*Bobin)*Insignia 49), Warigal: (WW 15*Raven) and (W1*MMC)/W1/10: (Warigal* ((WW22A*Mengavi)*Crim)). Warigal is therefore a parent of (W1*MMC)/W1/10.

Field experiment

During 1987 Halberd, Warigal and (W1*MMC)/W1/10 were grown at Two Wells, a site having high concentrations of boron in the sub-soil (Cartwright *et al.*, 1987). The experimental design consisted of a completely randomised block of nine replicates. The genotypes were grown as paired plots (plot size 4 rows × 4.2 m). Ten whole shoots were harvested from one plot of each pair when plants were at the boot stage. The grain was harvested from the adjacent plot at maturity and weighed. The concentration of boron in shoots at boot stage and in mature grain was determined by the methods described below.

Pot experiment

The soil used was a surface sample from a red-brown earth, Typic Haploxeralf (Soil Survey Staff, 1975). Boron, as boric acid, was applied in solution at concentrations of 0, 20 and 60 mgB kg^{-1} soil (treatments designated as B0, B20 and B60, respectively). Hot water extractable boron concentrations (Aitken *et al.*, 1987) at these treatments were 2, 14 and 43 mg kg^{-1} respectively. Soil (900 g) was weighed into 100 mm diameter pots lined with polythene bags. The pots were maintained at 70 % of field capacity (46 % w/w) by watering to weight with de-ionised water. Two seeds were sown per pot and thinned to one plant, two weeks after emergence.

The experiment consisted of a split-plot design with six replicates. The genotypes and boron treatments were combined factorially and assigned to plots for three harvest dates. Development of plants was recorded non-destructively by measuring the heights of plants to the tip of the youngest expanded blade (YEB) four and six weeks after sowing, and by noting the time from sowing to commencement of tillering, first node visible, boot stage and ear emergence of the main shoot (Zadok's stages 21, 31, 43 and 50 respectively). Symptoms of boron toxicity were scored eight weeks after sowing for the youngest, the second youngest and the third youngest blades (YEB, YEB + 1 and YEB + 2, respectively) on the main shoot. Plants were harvested individually as they

attained specific growth stages — first node visible, boot stage and maturity — dried at 85 °C for 48 hours and weighed. The mature plants were hand threshed and grain weight and number determined.

Determination of boron concentrations

Oven-dried plant samples (straw only for mature harvest of the pot experiment) were ground in a stainless steel mill. Boron concentrations in one gram sub-samples of ground material, and whole grain, were determined by inductively coupled plasma spectrometry (Zarcinas and Cartwright, 1983) following digestion in nitric acid (Zarcinas, 1984).

General applicability of selected parameters

Responses to high concentrations of boron which were found to discriminate between genotypes in this experiment were tested against a group of 20 South Australian wheat cultivars which were grown in a pot experiment with treatments of 0, 50 and 100 mg kg^{-1} applied boron (designated as B0, B50 and B100, respectively) and under high boron conditions in the field at Two Wells in 1985 (Paull *et al.*, unpublished).

Results

Field trial

Halberd and Warigal had similar grain yields, however the yield of (W1*MMC)/W1/10 was significantly less than the other genotypes (Table 1). The three genotypes were ranked similarly for concentration of boron in whole shoots at maturity and the concentration of boron in grain at maturity. In both cases Halberd had significantly lower boron concentrations in shoots at boot stage and in grain at maturity than Warigal, which in turn was significantly lower than (W1*MMC)/W1/10. The correlation between the concentration of boron in shoots at boot stage and in grain at maturity, over all plots, was $r = 0.92$ ($P < 0.001$).

Table 1. Grain yield and concentration of boron in whole shoots at boot stage and in grain at maturity for three wheat genotypes grown under high boron conditions in the field

Genotype	Grain yield (g $plot^{-1}$)	Boron conc (mg kg^{-1})	
		Boot stage	Grain
Halberd	521	25	2.6
Warigal	523	50	3.9
(W1*MMC)/W1/10	354	76	6.3
LSD ($P < 0.05$)	78	7	0.5

Pot experiment

Tissue boron concentrations. The concentrations of boron in whole shoots, straw and grain increased with applied boron (Table 2). Halberd had the lowest concentration of boron in the shoots at the B20 and B60 treatments for the first node and boot stage harvests and in the straw at maturity. The response for Warigal varied from being similar to significantly greater than Halberd, while (W1*MMC)/W1/10 had the highest concentrations. The concentration of boron in the shoots for each of the three genotypes in the B20 and B60 treatments was similar for the first node and boot stage harvests. The concentration in the straw at maturity was greater than at the earlier harvests and the relative differences in concentrations between genotypes were less at this stage. Halberd had significantly higher boron concentrations in the grain for the B20 and B60 treatments, while Warigal and (W1*MMC)/W1/10 were similar.

Yield. There was no significant difference in dry matter yield, between the three genotypes at the B0 treatment, for the first node, boot stage or maturity harvests, but the grain yield of Halberd was less than the two semi-dwarfs (Fig. 1). At the B20 treatment for the first node harvest the yields of both Warigal and (W1*MMC)/W1/10 were significantly reduced, while Halberd was not affected, however at B60 the yields of all genotypes were reduced. At this treatment Halberd yielded significantly more than Warigal, which in turn yielded significantly more than (W1*MMC)/W1/10. At the boot stage, the yields of all genotypes were reduced by the B20 and B60 treatments, but at B60 Halberd yielded significantly more than the other genotypes. At maturity, plants in the B20 treatment produced

Table 2. Concentration of boron in whole shoots at the first node and boot stages, and in straw and grain at maturity for three wheat genotypes grown in pots

Harvest Development	Treatment B (mg kg^{-1})	Genotype			LSD ($P < 0.05$)
		Halberd	Warigal	(W1*MMC)	
First node	0	6	8	7	n.s.
	20	68	84	137	32
	60	402	483	1077	179
Boot stage	0	6	5	4	n.s.
	20	78	81	123	30
	60	371	499	805	200
Straw	0	14	17	17	n.s.
	20	129	153	245	37
	60	862	918	1062	121
Grain	0	1.1	1.2	1.3	n.s.
	20	4.1	3.1	3.4	0.6
	60	21.5	17.3	16.2	2.9

more total dry matter than the B0 plants for both Halberd ($P < 0.01$) and Warigal ($P < 0.05$), while (W1*MMC)/W1/10 reacted similarly at the two treatments. The yield of Halberd was unaffected by the B60 treatment, relative to the control, but Warigal and (W1*MMC)/W1/10 were significantly reduced with (W1*MMC)/W1/10 being the more sensitive of the two.

Grain yields reflected the response of the whole shoots for all genotypes and treatments except (W1*MMC)/W1/10 at the B60 treatment (Fig. 1b). In this case grain yield was more sensitive than total biomass production. The reduction in grain yield by the B60 treatment was due to the combined effects of reduced spikelets per ear and reduced ears per plant.

The relative yields of Warigal and (W1*MMC)/W1/10 in the B20 and B60 treatments, expressed as a percentage of Halberd at the same treatment, increased for the later harvests (Fig. 1c). For example, (W1*MMC)/W1/10 in the B20 treatment yielded 75, 86 and 92 percent of Halberd for the first node, boot stage and maturity harvests, while in B60 the relative yields were 48, 61 and 68 percent, respectively. For screening at a single treatment the differences between the tolerant and sensitive genotypes were maximised at the earlier growth stages.

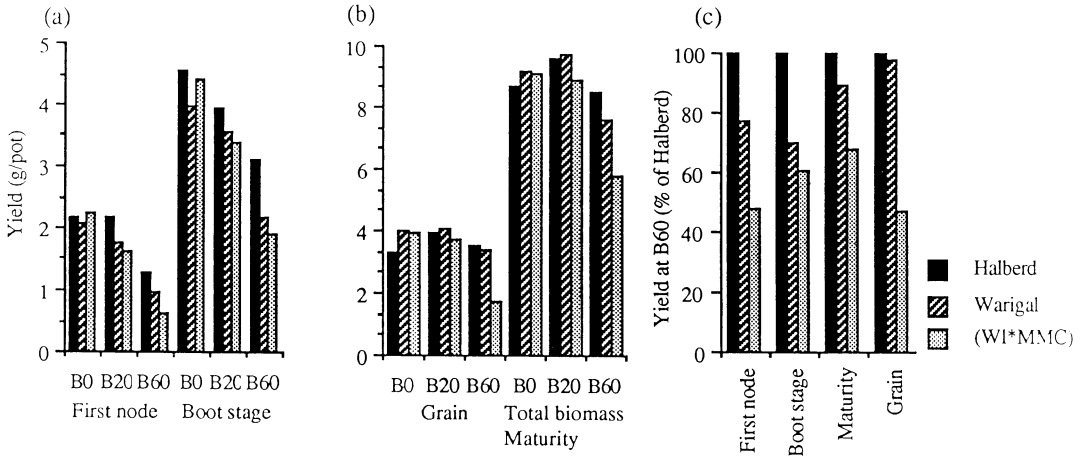

Fig. 1. (**a**) Total dry matter yield at the first node and boot stage harvests, (**b**) yield of grain and total dry matter, and (**c**) yield at the B60 treatment expressed as a percentage of Halberd at B60.

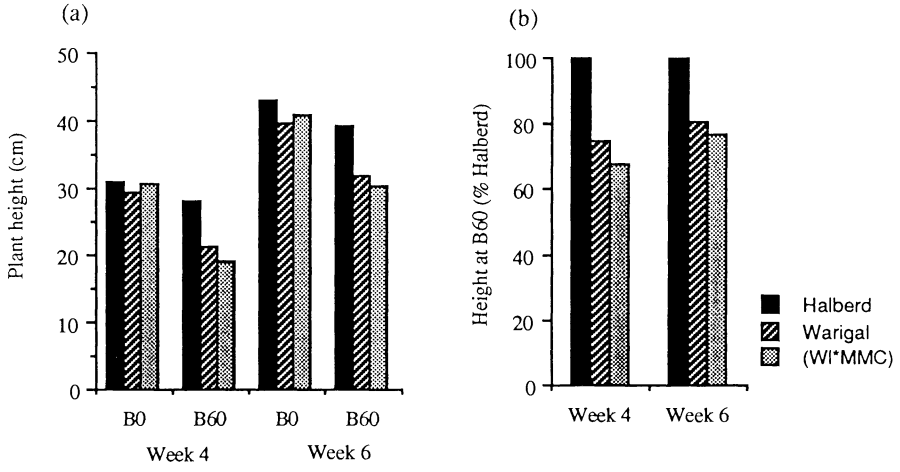

Fig. 2. Effect of boron on plant height four and six weeks after sowing. (**a**) Height at the B0 and B60 treatments, and (**b**) height at the B60 treatment expressed as a percentage of Halberd at B60.

Plant height. Plant height to the tip of the YEB at the B20 treatment did not result in a reduction in plant height, relative to the control treatment, however heights of all genotypes were reduced by the B60 treatment (Fig. 2a). In the B60 treatment the plant heights for Halberd were greater than for Warigal and (W1*MMC)/W1/10 on both occasions. The relative difference in plant height between genotypes, expressed as a percentage of Halberd in the B60 treatment, was greater four weeks than six weeks after sowing (Fig. 2b).

Delay in development. Plant development as measured by the time from sowing to specific growth stages was delayed by the B60 treatment (Fig. 3). Tillering of Halberd was delayed five days, Warigal 14 days and (W1*MMC)/W1/10 16 days by the B60 treatment, relative to the control. The B60 treatment did not delay the first node, boot stage or ear emergence for Halberd or Warigal, however the development of (W1*MMC)/W1/10 was consistently delayed by approximately 14 days. The B20 treatment had no significant effect on the rate of development relative to the control.

Symptoms. The symptoms of boron toxicity of wheat have been described (Gupta, 1971; Paull *et al.*, 1988) and consist of chlorotic and necrotic

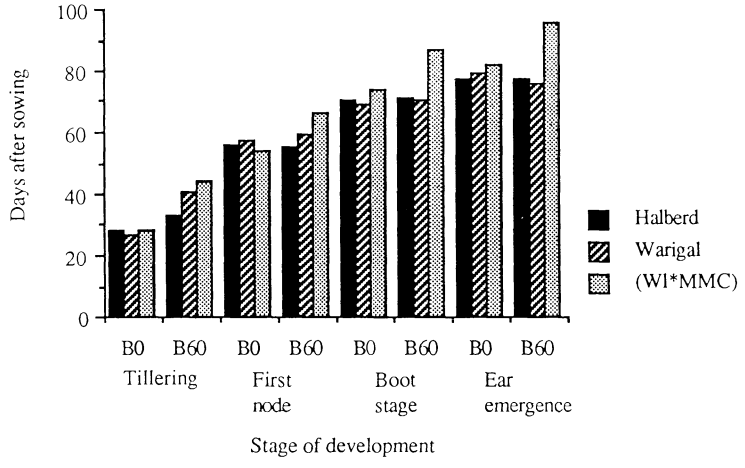

Fig. 3. Time from sowing to specific growth stages at the B0 and B60 treatments.

Table 3. Expression of symptoms of boron toxicity for the YEB, YEB + 1 and YEB + 2 of the main shoot eight weeks after sowing for three wheat genotypes grown in pots

Treatment B (mg kg^{-1})	YEB			YEB + 1			YEB + 2		
	Hlbd	Wgl	(W1*MMC)	Hlbd	Wgl	(W1*MMC)	Hlbd	Wgl	(W1*MMC)
0	9	9	9	9	9	9	9	9	9
20	9	8	8	8	8	7	7	7	6
60	7	7	5	6	6	4	6	6	3

Visual rating‡	Description of B damage
9	no visual symptoms
7	tip necrosis (1 cm)
5	1/4 leaf blade severe chlorosis with > 1 cm tip necrosis
3	1/2 leaf blade necrosis
1	leaf dead.

Adapted from Kluge and Podlesak (1985)

lesions developing from the tips of the leaves, along the margins, into the mid-rid and towards the base. The expression of symptoms indicative of boron toxicity for the YEB, YEB + 1 and YEB + 2 of the main shoot were rated on a scale from 1 (leaf dead) to 9 (no symptoms) eight weeks after sowing (Table 3) using a scale adapted from that of Kluge and Podlesak (1985). There were no symptoms of boron toxicity in the B0 treatment, while symptoms for the YEB in the B20 treatment were similar between genotypes and consisted of tip chlorosis. The symptoms for the YEB + 1 and YEB + 2 were similar for Halberd and Warigal, however (W1*MMC)/W1/10 was affected to a greater degree. At B60 the symptoms for Halberd and Warigal were the same for all leaves, while (W1*MMC)/W1/10 was again more severely affected. The degree of leaf damage increased for successively older leaves for the three genotypes in the B20 and B60 treatments.

General applicability of selected parameters

The ranking of cultivars for relative height (height at B100/height at B0) three weeks after sowing, in the pot experiment, was consistent with the ranking for grain yield and concentration of boron in the grain for the 20 cultivars grown in the field at a high boron site (r = 0.72 and − 0.59, respectively). The concentration of boron in shoots at boot stage in the B50 treatment was also significantly correlated with boron concentration in both shoots and grain for the field experiment (r = 0.59 and 0.69, respectively). The total dry matter production at maturity at the B100 treatment, however, was not significantly correlated with grain yield in the field.

Tillering was delayed by increasing boron treatments in the pot experiment and at B100 only 8 of the 20 cultivars produced tillers, although all developed to maturity. The four cultivars (Halberd, Dagger, Spear and Olympic) which had the lowest concentrations of boron in the shoots and grain at Two Wells produced tillers at B100, while the other four cultivars which produced tillers at B100 had an extended vegetative stage and did not reach the boot stage until three weeks after the other cultivars.

Discussion

Different methods for assessing boron tolerance are necessary with segregating and non-segregating generations. For fixed genotypes of an inbreeding species sufficient seed can be produced to allow replicated experiments to be conducted at a number of treatments. Critical levels and the rate of yield reduction at treatments above the critical level can be derived. The response to high boron treatments can also be compared to the control treatment. On the other hand, each genotype in a segregating generation such as an F_2 or BC_1 is represented only once. Screening must therefore be conducted at a single treatment without replication, and the response of individuals compared to a standard variety, for example one of the parents. For both methods of screening the response measured in the glasshouse should predict the response to high concentrations of boron in the field.

Three genotypes were grown in a field experiment to confirm previous observations of their responses to high concentrations of boron in the field. While in this experiment Halberd and Warigal had similar grain yields and (W1*MMC)/W1/10 significantly less, in the absence of boron toxicity it would be expected that the grain yield of Halberd, the only tall wheat, would be lower than the two semi-dwarfs. In a previous trial in an adjacent paddock, when the concentrations of boron in the shoots, at boot stage, for Halberd and Warigal were 48 and 77 mg kg^{-1}, respectively indicating a higher level of boron toxicity, Halberd (363 g/plot) outyielded Warigal (236 g/plot) (Cartwright *et al.*, 1987). The concentrations of boron in shoots and grain, for Halberd, are consistently less than for Warigal which is lower than (W1*MMC)/W1/10 (Table 1). A highly significant regression between concentration of boron in the shoots and grain yield has been reported for a group of 20 diverse genotypes (Rathjen *et al.*, 1987), therefore results of two field experiments confirm that Halberd is the most tolerant, Warigal intermediate and (W1*MMC)/W1/10 the most sensitive of these three genotypes to high concentrations of boron under field conditions.

The ranking of the three genotypes for concentration of boron in shoots at the first node and boot stages, and in the straw at maturity and for dry matter yield at the three harvest times was the same in the pot experiment as for concentration of boron in shoots at boot stage, and grain yield in the field. The greatest difference in concentration of boron in the shoots between the three genotypes occurred at the first node and boot stage harvests. Between these two harvests the concentration of boron in the shoots was constant for all genotypes in the B20 and B60 treatments. Therefore, between these growth stages plants could be harvested for tissue analysis at the same time without having to allow for differences in the stage of development. The concentrations of boron in shoots in the pot experiment were much higher than those obtained in the field, however there was no reduction in grain yield in the B20 treatment for any reduction for wheat genotypes having from 20 to 125 mg kg^{-1} in whole shoots at boot stage when grown under high boron conditions in the field. The critical concentration of boron in shoots therefore appears to vary between field and glasshouse conditions.

In contrast to the field situation where the concentration of boron in grain appears to be a reliable indicator of the concentration of boron in whole shoots at boot stage (Cartwright *et al.*, 1987), differences between genotypes in the concentration of boron in the grain did not reflect the concentrations of boron in shoots for the pot experiment. Both straw and grain are inappropriate tissues to analyse to assess the tolerance of a genotype to high concentrations of boron when grown in pots.

The difference in dry matter production between genotypes in the B20 and B60 treatments was greater at the earlier harvests than at maturity, however the ranking of genotypes was consistent between growth stages. The interpretation of the dry matter and grain yield data is confounded by the delay in development in the B60 treatment as plants were harvested as they attained the designated stage of growth, rather than all at the same time. The development of (W1*MMC)/W1/10 was delayed to a greater extent by the boron treatments than were Halberd and Warigal. Therefore, the differences in yield between the B60 and B0 treatments and between genotypes in the B60 treatment would have been increased by harvesting all plants at the same time.

The non-destructive measurements ranked the genotypes in the same order as the field experiment. Boron toxicity has been reported to reduce seedling height for a single genotype (Khudairi, 1961), and in this experiment height proved to be an indicator of the vigour, or tolerance, of the genotypes at the B60 treatment. Halberd was more tolerant than Warigal and (W1*MMC)/W1/10 on the basis of time to tillering in the B60 treatment. The time from sowing to the later stages of development rates Warigal the same as Halberd. Since the time from sowing to the first node and later stages of development were rated on the basis of the main shoot, the contrasting response of Warigal between tillering and the later stages indicate that tillering is more sensitive to high concentrations of boron than the development of the main shoot. The rate of development of the main shoot from the first node stage to ear emergence in the B60 treatment was the same for (W1*MMC)/W1/10 as Halberd and Warigal despite the first node stage for (W1*MMC)/W1/10 at the B60 treatment being delayed 14 days relative to the other two genotypes.

The expression of symptoms of boron toxicity

increased with increasing concentrations of applied boron and for the older leaves. The maximum difference between genotypes for expression of symptoms occurred on the YEB + 2 in the B60 treatment, however the symptoms on the YEB and YEB + 1 for (W1*MMC)/W1/10 were severe enough to distinguish it from the other two. As Halberd and Warigal had similar concentrations of boron in shoots, a similar expression of symptoms is to be expected. In comparison, (W1*MMC)/W1/10 had higher concentrations of boron in the shoots and more severe symptoms of boron toxicity. Oertli and Kohl (1961) described symptoms of boron toxicity for a number of species and found that the concentrations of boron associated with chlorotic and necrotic tissue were consistent between species.

Parameters which discriminated between the responses of the three genotypes were found to be applicable to a wider range of cultivars and gave similar rankings in pots as in the field. Seedling height proved to be more reliable than yield at maturity in the B100 treatment as an indicator of the response of a cultivar to high boron conditions in the field. This would be due to differences in growth rates between cultivars and treatments. A moderately sensitive genotype may produce the same total biomass at maturity as a more tolerant genotype due to an extended vegetative phase. Screening at an early stage in development would therefore result in the greatest discrimination between tolerant and moderately tolerant genotypes. The concentration of boron in shoots was also consistent between pot and field experiments for the larger number of genotypes. Tolerance of high concentrations of soil boron has consistently been found to be associated with lower concentrations of boron in the shoots and grain (Cartwright *et al.*, 1987; Paull *et al.*, 1988; Rathjen *et al.*, 1987). As yield potential varies between genotypes and grain yield is affected by many factors other than boron toxicity, selection for tolerance of high concentrations of boron per se under field conditions may best be achieved by tissue analysis.

The ranking of genotypes for tolerance to high concentrations of boron was consistent between the field and the early growth stages in pots, and also with our previous reports (Cartwright *et al.*, 1987; Paull *et al.*, 1988). Tolerance to boron is expressed early in development and the maximum differences in growth and concentrations of boron in the tissues, between the tolerant and sensitive genotypes grown in pots, occurred at the earliest sampling times. In pots, the relative tolerance of the genotypes was much less apparent at later stages through compensatory growth associated with the delay in time to maturity, and possibly, through a change in the root distribution in relation to water and boron uptake, to avoid the effects of high levels of soil boron. Glasshouse screening can be adopted for evaluating the response of wheat to high concentrations of boron. Non-destructive measurements including seedling height, rate of development and symptom expression at high concentrations of applied boron can be used for selecting individuals in the segregating generations of a breeding program, while dry matter production and boron determination could also be applied to advanced generations and cultivars.

Acknowledgements

We acknowledge the financial support of the South Australian Wheat Industry Research Committee.

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Session 4

Genetic variation in symbiotic systems

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 373–379.
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PLSO PN 177

Variability of molecular nitrogen fixation and its dependence on plant genotype and diazotroph strains

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Key words: diazotroph strains, genotype, wheat, sugar beet, sunflower

Abstract

The effect of selected diazotrophs of the genus Azotobacter, Klebsiella, Escherichia, Derxia, Azospirillum and Beijerinckia on the production of dry root mass and above ground parts and the concentration and control of nitrogen in wheat, sugar beet and sunflower genotypes has been investigated. The summed-up positive and negative effects on each plant species and diazotroph strain show a significant variability in relation to plant genotype, as well as in relation to diazotroph strain. Namely, certain diazotroph strains had a particularly positive effect in association with the genotypes of one plant species, while in the genotypes of other plant species they gave a smaller number of positive effects of caused expressive negative effects. The associations show the specifities of diazotrophs in relation to plant species as well as in relation to genotype.

Introduction

In order to continue our investigations into the problems of genetic aspect of mineral nutrition the interaction between the plant genotypes and strains of microorganisms was studied (Sarić 1981; 1983; 1987).

Our former results indicate the variability of effect of diazotroph strains in maize in dependence on genotype and nitrogen nutrition (Sarić *et al.*, 1987a; 1987b; 1988). Although certain microorganisms are capable of fixing free nitrogen non-sybiotically, their use in crop production has been limited. The investigations aimed at establishing new species and strains of active nitrogen-fixing bacteria and improving the activity of known microorganisms and their use in growing certain plant genotypes provide the possibility for a more intensive utilization of atmospheric nitrogen.

Therefore, the emphasis of this study is on the interaction between the plant genotypes and individual strains of nitrogen-fixing bacteria and their combinations.

Materials and methods

The seed of different cultivars (genotypes) of wheat (NS-Rana, Balkan, Yugoslavia, Zvezda, Žitnica), sugar beet (NS-701/013, NS-H-92, Perla, NS-HX-11, PN-Mono-4) and sunflower (RHA-26, NS-H-26-RM, L-1, L-22, NS-H-43, and R-SNRF) was sown in sterilized sand and inoculated with the strains of nitrogen-fixing bacteria belonging to six different genera, as well as to their combinations:

1. Azotobacter strain 20
2. Escherichia strain 32
3. Klebsiella strain 39
4. Derxia strain 4
5. Beijerinckia strain 4
6. Azospirillum strain 2
7. Combination of eminent Azotobacter strains (2, 3, 5, 8, 10, 16, 20)
8. Combination of eminent Escherichia strains (7, 32)
9. Combination of eminent Klebsiella strains (39, 54)
10. Combination of eminent Derxia strains (4, 8)

11. Combination of eminent Beijerinckia strains (2, 4)
12. Combination of eminent Azospirillum strains (2, 4)
13. Azotobacter strain 2
14. Azotobacter strain 5
15. Azotobacter strain 10
16. Azotobacter strain 16
17. Azotobacter strain 8
18. Combination 1–6
19. Combination 7–12
20. Control

The plants were grown in a greenhouse in sand cultures for 30 days. Noninoculated plants served as the control. The plants were irrigated with distilled water. The dry mass and concentration and contents of nitrogen in shoot and root were determined.

The summed-up results of positive and negative effects on the dry mass production, concentration and contents of nitrogen in root and above-ground plant part for all genotypes of certain plant species, *i.e.* for five genotypes in wheat and sugar beet and six genotypes in sunflower are presented in Tables (1, 2, 3). Also, the summed-up results of positive and negative effects for all three species for root and above-ground plant part are shown for each diazotroph variant (Table 5). All this was carried out on the basis of statistically calculated results tested by LSD with 5 and 1%.

Results

Dry matter

Table 1 shows that in wheat roots, the largest number of positive effects was obtained (53), somewhat smaller in sugar beet (16), and the smallest in sunflower (5). However, for the above-ground parts, the largest number of positive effects was obtained in sugar beet (59), then in wheat (36) and sunflower (27). The smallest number of negative effects in root was obtained in sugar beet (3), then in sunflower (10), while the largest number was in wheat (12). In above-ground parts of sugar beet there were no negative effects, in sunflower the number was 4, while in wheat 8. The total number of positive effects for the dry matter mass was the largest in wheat (89), then in sugar beet (75) and the smallest in sunflower (32).

However, the phenomenon of variation in the efficiency of diazotrophs in dependence on genotype within the plant species is very significant. The best results were obtained in the wheat cultivar NS-Rana (23) and the poorest in Zvezda (13). This ratio in sugar beet was 18:11 (the variety Perla : 7N-Mono-4), while in sunflower 9:1 (L-22 : R-SNRF).

Nitrogen concentration

Table 2 shows that in roots the largest number of positive effects was obtained in sugar beet (20), sunflower (11), and the smallest in wheat (5). However, in the above-ground part the largest number of positive effects was found in wheat (38), then in sugar beet (16) and sunflower (7) only. The largest number of negative effects in root was also obtained in wheat (33), while in sugar beet and sunflower only in two cases each. The number of negative effects in the above-ground parts was the smallest in sugar beet (2), then in wheat (4), while the largest number was found in sunflower (23). The total number of positive effects in nitrogen concentration was in wheat (43), then in sugar beet (36) and finally in sunflower (18).

This parameter shows that the analysed genotypes also behaved differently. Thus, in wheat the largest number of positive effects was found in the cultivar Zvezda (16) and the smallest number in the cultivar NS-Rana (5). In sugar beet this ratio was 10:2, while in sunflower 5:2 in dependence on the genotype.

Nitrogen content

Table 3 shows that the largest number of positive effects in root was obtained in sugar beet (30), then in wheat (27) and sunflower (7). However, in the above-ground part the positive effect was obtained in significantly more cases: in wheat (60), sugar beet (59) and in sunflower (24).

The number of negative effects in root was the smallest in sugar beet (1), then in wheat (10) and sunflower (14). In the above-ground part of wheat and sugar beet there were no negative effects, and in sunflower only one. The total number of positive

Table 1. Sum of positive and negative effect caused by diazotrophs on the mass of root and above-ground part of wheat, sugar beet and sunflower genotypes

		Wheat genotype						Sugar beet genotype						Sunflower genotype						
		1	2	3	4	5	Total	1	2	3	4	5	Total	1	2	3	4	5	6	Total
Root	+	8	16	13	5	11	53	1	5	4	0	6	16	3	0	0	2	0	0	5
	−	3	3	3	3	0	12	1	0	1	1	0	3	0	4	5	1	0	0	10
Above-ground part	+	15	6	1	8	6	36	10	7	14	16	12	59	4	6	4	7	5	1	27
	−	1	3	2	1	1	8	0	0	0	0	0	0	0	1	1	1	1	0	4
Total	+	23	22	14	13	17	89	11	12	18	16	18	75	7	6	4	9	5	1	32
	−	4	6	5	4	1	20	1	0	1	1	0	3	0	5	6	2	1	0	14

Table 2. Sum of positive and negative effects caused by diazotrophs on nitrogen concentration in root and above-ground part of wheat, sugar beet and sunflower genotypes

		Wheat genotype						Sugar beet genotype						Sunflower genotype						
		1	2	3	4	5	Total	1	2	3	4	5	Total	1	2	3	4	5	6	Total
Root	+	1	1	1	2	0	5	5	0	3	3	9	20	2	3	2	1	1	2	11
	−	8	8	0	14	3	33	0	2	0	0	0	2	2	0	0	0	0	0	2
Above-ground part	+	4	9	5	14	6	38	5	2	5	3	1	16	1	2	1	1	1	1	7
	−	4	0	0	0	0	4	0	2	0	0	0	2	3	2	1	4	5	8	23
Total	+	5	10	6	16	6	43	10	2	8	6	10	36	3	5	3	2	2	3	18
	−	12	8	0	14	3	37	0	4	0	0	0	4	5	2	1	4	5	8	25

Table 3. Sum of positive and negative effects caused by diazotrophs in nitrogen contents in root and above-ground part of wheat, sugar beet and sunflower genotypes

		Wheat genotype						Sugar beet genotype						Sunflower genotype						
		1	2	3	4	5	Total	1	2	3	4	5	Total	1	2	3	4	5	6	Total
Root	+	3	12	8	0	4	27	8	5	6	2	9	30	4	2	0	1	0	0	7
	−	2	2	2	4	0	10	0	0	1	0	0	1	0	5	4	2	3	0	14
Above-ground part	+	16	11	12	15	6	60	9	7	15	14	14	59	0	8	2	7	5	2	24
	−	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Total	+	19	23	20	15	10	87	17	12	21	16	23	89	4	10	2	8	5	2	31
	−	2	2	2	4	0	10	0	0	1	0	0	1	0	6	4	2	3	0	15

Table 4. Sum of positive and negative effect caused by diazotrophs in root and above-ground part of wheat, sugar beet and sunflower genotypes

		Wheat genotype						Sugar beet genotype						Sunflower genotype						
		1	2	3	4	5	Total	1	2	3	4	5	Total	1	2	3	4	5	6	Total
Root	+	12	29	22	7	15	85	14	10	13	5	24	66	9	5	2	4	1	2	23
	−	13	13	5	21	3	55	1	2	2	1	0	6	2	9	9	3	3	0	26
Above-ground part	+	35	26	18	37	18	134	24	16	34	33	27	134	5	16	7	15	11	4	58
	−	5	3	2	1	1	12	0	2	0	0	0	2	3	4	2	5	6	8	28
Total	+	47	55	40	44	33	219	38	26	47	38	51	200	14	21	9	19	12	6	81
	−	18	16	7	22	4	67	1	4	2	1	0	8	5	13	11	8	9	8	54

effects was found in sugar beet (89), wheat (87) and the smallest number in sunflower (31).

The genotypes also differed much in this parameter. The largest number of positive effects in wheat was obtained by the cultivar Balkan (23), and the smallest in the cultivar Žitnica (10). This ratio in sugar beet was 23:12, and 10:2 in sunflower.

The total effect for root and above-ground parts

Table 4 shows that the largest number of the total positive effects for root was in wheat (85) then in sugar beet (66) and sunflower (23). The order for the above-ground part was the same, but the number of positive effects was much larger. Wheat and sugar beet (134) took first place, and sunflower was significantly lower (58). Negative effects for roots were also obtained in most cases in wheat (55), then in sunflower (26) and the fewest in sugar beet (6).

The largest number of negative effects for the above-ground part was found in sunflower (28), then in wheat (12) and sugar beet (2). The total number of positive effects was the largest in wheat (219), then in sugar beet (200) and the smallest in sunflower (81).

As far as the total effects are concerned, the genotypes also differed significantly among themselves. In wheat the total rumber of positive cases was the largest in the cultivar Balkan (55) and the smallest in the cultivar Žitnica (33), while this ratio in sugar beet was 51:26 and 21:6 in sunflower.

The efficiency of different strains of diazotrophs

The summed-up results show (Table 5) that the analysed diazotraph variants differed significantly among themselves in efficiency of certain plant species. The most efficient strains in wheat were Azotobacter 10, Azotobacter 8, then Klebsiella 39 and Azotobacter 16. Contrary to these strains, Beijerinckia 4 caused the largest number of negative effects, as well as Derxia 4. It is characteristic that the strains Azotobacter 10 and 8 did not cause a single negative effect.

In sugar beet the best effect was obtained with the mixture Azospirillum 2 and 4, then Azotobacter 20 and Beijerinckia 4. We may say that these strains did not have negative effects.

In contrast to wheat and sugar beet, the efficiency of the analysed diazotrophs was the lowest in sunflower. The mixture *Escherichia* had the best effect, but in this variant the number of negative effects was the largest. In a number of cases in this plant species, certain variants of diazotrophs caused the same number of positive as well as negative effects.

In wheat the most effective inoculation was obtained with Azotobacter 10 which gave 20 positive and 0 negative effects out of 30 possible cases (5 cultivars × 3 parameters × 2 organs). In sugar beet 13 variants of inoculation did not have a single negative effect. In sunflower, much larger number of effects was obtained at the level of control, showing that the effect of diazotrophs was weaker in this plant species.

Discussion

Our results show that the strains of diazotrophs and their combinations affect the increase or decrease of dry mass, concentration and nitrogen contents in root and above-ground part of wheat genotypes, sugar beet and sunflower. According to the summed-up positive effects, wheat takes the first place (219:67). But, the number of negative effects is taken into consideration, sugar beet is greater (200:80). From the positive and negative effects observed the diazotrophs had the lowest efficiency in sunflower. This does not mean that strains of diazotrophs in this species would not produce better results.

In all plant species, the effect of diazotrophs varied in dependence on genotypes, pointing out the fact that all genotypes do not form equal conditions for daizotrophs.

Some authors, who inoculated wheat with *Azospirillum* spp., succeeded in increasing the yield (Millet and Feldman, 1984; Reynders and Vlassak, 1982). Also, inoculation of wheat plants increased nitrogen fixation (Rennie *et al.*, 1983), while some others (Lethbridge and Davison, 1983) obtained an insignificant increase of nitrogenase activity in wheat, in absence of carbohydrates. The weak nitrogenase activity in wheat by inoculation of Azospirillum was also obtained (Kapulnik *et al.*,

Table 5. Summed-up positive and negative effects in root and above-ground part of wheat, sugar beet and sunflower for each diazotroph variant

Organs		Vatiants of used diazotrophs in trials																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
		Wheat																			
Root	+	5	5	5	2	3	6	3	3	4	4	1	5	4	5	11	6	5	4	4	85
	−	2	4	2	7	8	2	1	4	2	3	5	2	3	3	0	1	0	3	3	55
Above-ground	+	2	6	9	8	7	8	6	6	6	9	10	4	7	5	9	8	11	4	7	134
part	−	1	1	0	2	3	0	1	1	0	0	1	0	0	1	0	0	0	1	0	12
Total	+	8	12	14	10	10	14	9	9	10	13	11	9	11	10	20	14	16	8	11	219
	−	4	5	2	9	11	2	2	5	2	3	6	2	3	4	0	1	0	4	3	67
		Sugar beet																			
Root	+	5	5	2	4	5	3	2	2	2	3	1	9	2	3	5	3	3	2	5	66
	−	0	3	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	6
Above-ground	+	13	3	8	5	11	10	6	1	10	7	6	10	5	5	3	6	8	7	10	134
part	−	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2
Total	+	18	7	11	9	16	13	8	3	12	10	7	19	7	8	8	9	11	9	15	200
	−	0	3	0	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	8
		Sunflower																			
Root	+	2	2	2	4	1	1	0	3	3	1	1	0	1	0	1	1	0	0	0	23
	−	0	0	1	1	1	2	0	7	3	0	1	0	0	0	0	1	3	3	3	26
Above-ground	+	6	4	6	0	1	0	3	9	6	3	3	2	2	1	2	4	1	3	2	58
part	−	1	2	1	1	0	3	2	4	0	1	2	2	2	1	3	2	1	0	0	28
Total	+	8	6	8	4	2	1	3	12	9	4	4	2	3	1	3	5	1	3	2	81
	−	1	2	2	2	1	4	2	11	3	1	3	2	3	1	3	3	4	3	3	54

1985) under field conditions. Our results also show that Azospirillum strains are less efficient in wheat, but were highly efficient in sugar beet. In wheat, higher efficiency and universality were shown by the strains Azotobacter 10 and 8, as well as the strains from the genus Klebsiella, which caused higher nitrogen contents in wheat genotypes than Azospirillum strains by nitrogen fixation.

In our work, the strains of the genus Azotobacter in certain genotypes of wheat, sugar beet and sunflower, as well as maize showed excellent activity in nitrogen fixation (Sarić *et al.*, 1987; a; b; 1988). Thus, we consider that the genus Azotobacter deserves more attention in diazotroph selection, since it has not only considerable activity in nitrogen fixation, but also the ability to synthesize growth substances. It should be also stressed that diazotroph selection should be carried out for each genotype in order to find strains adapted to the conditions of genotype rhizosphere. Though, it would be ideal to find more universal strains, i.e. effective for a larger number of genotypes.

The question to be answered is: what is the cause of variation in efficiency of plant genotypes and microorganism strains when free nitrogen-fixing bacteria are applied? It may be assumed that the relationship between the plant genotype and the strain of microorganisms depends on the following characteristics of partners in the system:

Quantitative and qualitative composition of root exudates of a certain genotype (amount of exuded organic carbon-sugars, organic acids, amino acids, etc.).

Specificity of metabolism of a microorganism strain as dependent on the synthesis of certain compounds (acids: lactic, propionic, butyric, pyruvic, succinic, and malic; alcohols: ethanol, methanol, butanol, propanol, and isopropanol; compounds of indole and phenols).

Ability of a microorganism strain and a plant genotype to synthesize phytohormones (gibberellin, auxin, cytokinine, abscisic acid, *etc.*).

Ability of a microorganism strain to synthesize inhibitors (antibiotics, phenols, various toxins *etc.*).

Influence of the system (plant genotype—microorganism strain) on an increased strain competitive ability relative to other microorganisms from the rhizosphere and epiphytic seed microflora.

Ability of a microorganism strain from the

system to colonize root surface and penetrate into plant tissue.

Influence of the system (genotype—strain) on changes of the ecological factors of the rhizosphere (pH, rH_2, pO_2, CO_2, *etc.*).

Specificity of a plant genotype related to nitrogen uptake and transport, *i.e.* different concentration and contents of nitrogen in root and shoot tissue.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 381–384.
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PLSO PN 178

Iron deficiency in chickpea in the Mediterranean region and its control through resistant genotypes and nutrient application

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Key words: chickpea, foliar application of $FeSO_4$, genetic differences in resistance to Fe-deficiency, germplasm Fe-deficiency, inheritance of resistance

Abstract

Iron-deficiency chlorosis is commonly observed in some genotypes of chickpea (*Cicer arietinum* L.) in the Mediterranean region of West Asia and North Africa when grown on calcareous soils. An evaluation of 3267 germplasm lines of kabuli-type chickpea for iron-deficiency chlorosis on the calcareous soil (calcium carbonate content ~20%, pH ~8.5) of the principal research station of the International Centre for Agricultural Research in the Dry Areas (ICARDA) at Tel Hadya, northern Syria, revealed that most of the lines were tolerant while only 25 lines showed susceptibility. Foliar spray of 0.5% $FeSO_4$ at the onset of chlorosis was effective in correcting the symptoms but did not result in significant increase in crop yield.

Studies on the inheritance of resistance to iron-deficiency chlorosis revealed that the resistance was dominant and is governed by a single gene.

To ensure elimination from the breeding material of chickpea genotypes inefficient in Fe-use on the calcareous soils of the Mediterranean region, a negative selection for Fe-deficiency chlorosis in the segregating populations in the field has proved effective. The method for field screening of large number of breeding lines and germplasm is described.

Introduction

Iron-deficiency chlorosis is a complex physiological disorder that generally occurs in crop plants grown on a high pH calcareous soil because of low availability of iron in the soil and inadequate uptake or because of an impairment of transport and metabolism of iron induced by other ions (Foy, 1983). Fe-deficiency chlorosis has been observed in several chickpea (*Cicer arietinum* L.) crops grown on high pH calcareous vertisols in India (Kannan, 1984; Napinder *et al.*, 1984; Sakal *et al.*, 1987; Saxena and Sheldrake, 1980; Saxena and Singh, 1977; Singh *et al.*, 1984; Singh *et al.*, 1986) and in the West Asia and North Africa (WANA) region (Halila, 1983; Saxena, 1980). Significant reduction in the yield of chickpea was observed in India when foliar spraying of ferrous sulphate was not carried out to correct the Fe-deficiency chlorosis (Sakal *et al.*, 1987; Saxena and Sheldrake, 1980; Singh *et al.*, 1984).

Materials and methods

Field experiments were conducted at the Tel Hadya Research Station of ICARDA in northern Syria (36°N, 362 m above sea) where the soil is chromic luvisol and uniformly calcareous with a calcium carbonate content of more than 20% (range 20.4–22.8%), pH (water) of about 8.5 (range 8.4–8.6), and organic carbon content of about 0.45% (range 0.41–0.54%). Winter sowing of chickpea can be done in the end of November or beginning of December with much profit of increased yield and water-use efficiency when compared to that of spring sown crop (Saxena, 1980).

Field observations with chickpea genotypes

grown for breeding work at Tel Hadya in the past showed that the duration for which the chlorosis persisted in the susceptible types varied with genotypes and season. Mostly the desi-type chickpea genotypes introduced from India showed chlorosis whereas the local kabuli-types native to WANA region showed little or no chlorosis. Since ICARDA maintains world collection of Kabuli-type chickpea, it was thought worthwhile to evaluate the reaction of this collection to the possible Fe-deficiency on the calcareous soils of ICARDA experimental station. Germplasm lines (a total of 3267 accessions) were grown in 1978/79 as 1 m long single-row plots and scored for Fe-deficiency chlorosis 35–40 days after sowing when the susceptible types showed severe chlorosis. The scoring was done on a 1–9 point scale were 1 = no Fe-deficiency chlorosis and 9 = all plants in a row showing severe chlorosis with the youngest leaves completely bleached and necrotic.

The method of screening involves growing the breeding material in a field with uniformly calcareous soil in early spring, as 1 m long single-row plot per line with a susceptible genotype planted as indicator and a check after every 9 test entries, and irrigating the crop at the 4–5 leaf stage (about 35–40 days after sowing) to create conditions conducive for the development of Fe-deficiency chlorosis. The evaluation of test entries is done when the susceptible check entry starts showing clear symptoms of Fe-deficiency.

A field trial was conducted during the 1981/82 season using two Fe-deficiency chlorosis susceptible desi-type genotypes (NEC 2305 and C 235) and a tolerant kabuli-type (ILC 482). Fe was sprayed on the foliage as 0.5% ferrous sulphate solution (500 L/ha) or equivalent amount as ferrous chloride and Fe-sequestrine at the start of the chlorosis in the most susceptible line (NEC 2305). The untreated check received only a water spray.

Two susceptible parents (ICCL 81131 and ICCL 81192) were crossed with a resistant parent (ILC 263) in the 1981/82 season at Tel Hadya. During the 1982/83 season these parents and their F_1's were grown to: (i) make back-crosses, (ii) advance the generation to F_2, and (iii) make fresh F_1's for each cross. In the 1983/84 season, the parents, the F_1's, the F_2's and the back-crosses were all grown on a uniformly calcareous soil in a randomized block design with three replications.

Results and discussion

An evaluation of 3267 germplasm accessions of kabuli-type chickpea revealed that a majority of these accessions were tolerant of Fe-deficiency and only 25 accessions exhibited Fe-deficiency chlorosis. Gowda and Smithson (1980) examined the variability in susceptibility to Fe-deficiency chlorosis in a predominantly desi chickpea material and reported wide variations in the genotypes for their susceptibility to Fe-chlorosis, which started 25–30 days after sowing and generally disappeared 10–15 days thereafter. Late maturing lines had higher frequency of susceptible lines than the early maturing ones and this difference was attributed to the rate of shoot and root growth of the early and late maturing types. Genotypic differences in Fe-deficiency chlorosis susceptibility have also been reported in chickpea on high pH calcareous soils at other locations in India (Malewar and Chhabda, 1979; Malewar *et al.*, 1982; Singh *et al.*, 1984; Singh *et al.*, 1986).

Half percent ferrous sulphate solution was most effective in correcting the Fe-deficiency chlorosis in susceptible and tolerant chickpea as the susceptible plants treated with this source were the first to recover. The untreated plants of the susceptible genotypes also recovered, but about 2 weeks later than those sprayed with ferrous sulphate. It appears, therefore, that the Fe-deficiency chlorosis in the early stage of crop growth in the susceptible genotypes does not leave a long lasting influence on crop growth as it occurs when the rate of growth is rather slow. As the temperatures rise and rate of growth increases the untreated susceptible genotypes are able to absorb adequate amount of Fe, perhaps from the larger soil volume explored by expanded root system (Malewar and Chhabda, 1979; Malewar *et al.*, 1982) to catch up in growth with the plants sprayed with ferrous sulphate. Results of the CIET in 1982/83 and 1983/84 at several locations in WANA were similar to those described above: Fe-deficiency chlorosis was rapidly corrected by spraying 0.5% ferrous sulphate solution but this did not result in significant increase in chickpea seed yield (ICARDA, 1985; 1986). Fe-deficiency chlorosis in WANA region may not therefore be a serious limitation to yield in the existing land races and cultivars of chickpea. These results of course contrast with those

Table 1. Number of Fe-deficiency chlorosis resistant and susceptible plants, Chi-square values (X^2) and probabilities of goodness of fit (P) to a ratio of 3:1 in the F_2 populations of crosses of resistant parent (ILC 263) with susceptible parents (ICCL 81192 and ICCL 81131), and to a ratio of 1:1 in the back-cross (BC) with a susceptible parent

Parent/Cross		Number of plants		X^2	*P*
		Resistant	Susceptible		
ICCL 81131	(P_1)	0	22		
ILC 263	(P_2)	20	0		
ICCL 81192	(P_3)	0	24		
$P_1 \times P_2$	F_1	17	0		
$P_1 \times P_2$	F_2	176	66	0.67	0.50–0.30
$(P_1 \times P_2) \times P_1$	BC	11	13	0.17	0.70–0.50
$(P_1 \times P_2) \times P_2$	BC	24	0		
$P_3 \times P_2$	F_1	15	0		
$P_3 \times P_2$	F_2	214	63	0.75	0.70–0.30
$(P_3 \times P_2) \times P_2$	BC	21	0		
$(P_3 \times P_2) \times P_3$	BC	16	13	0.31	0.70–0.50

obtained with desi-type chickpea in some of the high pH vertisols in India (Sakal *et al.*, 1987; Saxena and Sheldrake, 1980; Singh *et al.*, 1984; Singh *et al.*, 1986).

Although the kabuli type chickpea germplasm maintained at ICARDA has only a small percentage of accessions that show Fe-deficiency chlorosis, ICARDA's chickpea improvement programme uses very diverse genetic material to create large genetic variability and develop new recombinants. In this process, the Indian desi- and kabuli-types are also used for crossing. We, therefore, have to ensure that the elite genotypes being developed from these crossing programmes are free from Fe-chlorosis susceptibility. In order to be able to do so we have tried to study the inheritance of resistance to Fe-deficiency chlorosis using a resistant parent (ILC 263) and two susceptible parents (ICCL 81131 and ICCL 81192). The results of the evaluation of parents, F_1's, F_2's and the back-crosses for their Fe-deficiency chlorosis in the field are presented in Table 1. The X^2-test revealed the goodness of fit of 3 resistant to 1 susceptible ratio in the F_2 and 1 resistant to 1 chlorotic in the back-crosses of F_1's with the susceptible parents. It can, therefore, be concluded that the 'Fe-deficiency chlorosis resistance' is dominant over the 'susceptibility' and is controlled by a single gene. Similar results have recently been obtained at ICRISAT as well (Gowda and Rao, 1986). Fe-deficiency chlorosis resistance has been reported to be controlled by one major, dominant gene, with modifiers in oats (McDaniel and Brown, 1982), and by one dominant gene in tomato (Brown and Wann, 1982). In soybean, Weiss (1943) reported one major gene involved in Fe-deficiency, but Fehr (1982, 1983) found that this trait was quantitatively inherited and recommended that improved genetic resistance in soybean can be developed by recurrent selection.

Elimination of the Fe-inefficient chickpea genotypes has helped in the development of genetic stocks which show no Fe-deficiency chlorosis when grown in winter or spring season on soils with high pH (~ 8.5) and a calcium carbonate content of about 20%.

Improved Fe-efficiency in a particular genotype may accrue from one or more of the following attributes: ability to maintain a low pH in the root zone and release of some 'reductant' that converts F^{3+} to Fe^{2+}, more efficient transport of Fe from roots to tops and restricted transport of Mn and Ca as these cations may interfere with the metabolism of transported Fe (Foy, 1983). Tipton and Thowsen (1983) have observed higher rates of reduction of Fe^{3+} to Fe^{2+} by roots catalyzed by a cell wall enzyme in Fe-efficient soybean genotypes, a process different from the non-enzymatic reduction caused by root secreted reductant.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 385–390.
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PLSO PN 179

N_2 fixation by *R. japonicum* strains during vegetation of different soybean cultivars

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Key words: nitrogenase activity, nitrogen content, nodule formation, *Rhizobium japonicum* strain, soybean cultivar

Abstract

Experiments were conducted with soil cultures kept in a greenhouse in which soybean was grown to full grain maturity. In the course of soybean growth the dynamics of nitrogenase activity, nodular development and nitrogen fixation were monitored. Nitrogen fixation was most frequently correlated with the activity of nitrogenase, the formation of nodules and the total nitrogen content, but contrary results were also obtained. The nitrogenase activity and amount of fixed nitrogen kept changing during vegetation, depending on soybean cultivars and *Rhizobium japonicum* strains.

Introduction

Symbiotic relations between soybean plant and nodule bacteria have been examined by a number of authors (Israel *et al.*, 1986; Sarić and Sarić, 1959; Sarić, 1960). It has been noticed that certain strains show different efficiency with different cultivars (Sarić and Milić, 1981; Sarić and Krstić, 1982; Sarić and Fawzi, 1983).

Thus, the priority in the increased search for nitrogen fixation in soybean is to find out the most favorable combination cultivar-strain, whose mutual relations provide the highest nitrogen fixation. Also, it is known that there is a wide span in the fixation capacity of *R. japonicum* strains, thus the activity of nitrogenase could be an indicator of soybean nitrogen fixation (Hardy *et al.*, 1973).

It has been proved that the activity of nitrogenase changes during the day and during vegetative growth (Mederski and Streeter, 1977; Sloger *et al.*, 1975).

Methods

The experiments were carried out with six highly efficient strains. Their effect on soybean cultivars NS-6, NS-9, NS-10, and Hark were observed. Plants were grown in soil cultures in a greenhouse until they reached full maturity. In the course of the soybean growing season we followed the dynamics of nitrogenase activity, nodular development and nitrogen fixation, 3, 5, 7, 9, and 11 weeks after emergence. Nitrogenase activity was determined by the acetylene-ethylene method. The inoculation was performed at sowing and the soil was irrigated with distilled water. The experiment was established in five replications and two controls, one with sterilized and the other with nonsterilized soil. Nitrogen was determined by the Kjeldahl method.

Results

The results on the activity of nitrogenase, quantity of fixed nitrogen and forming of nodules in individual soybean cultivars are presented graphically and show certain characteristic relations of the soybean cultivars and the *R. japonicum* strains.

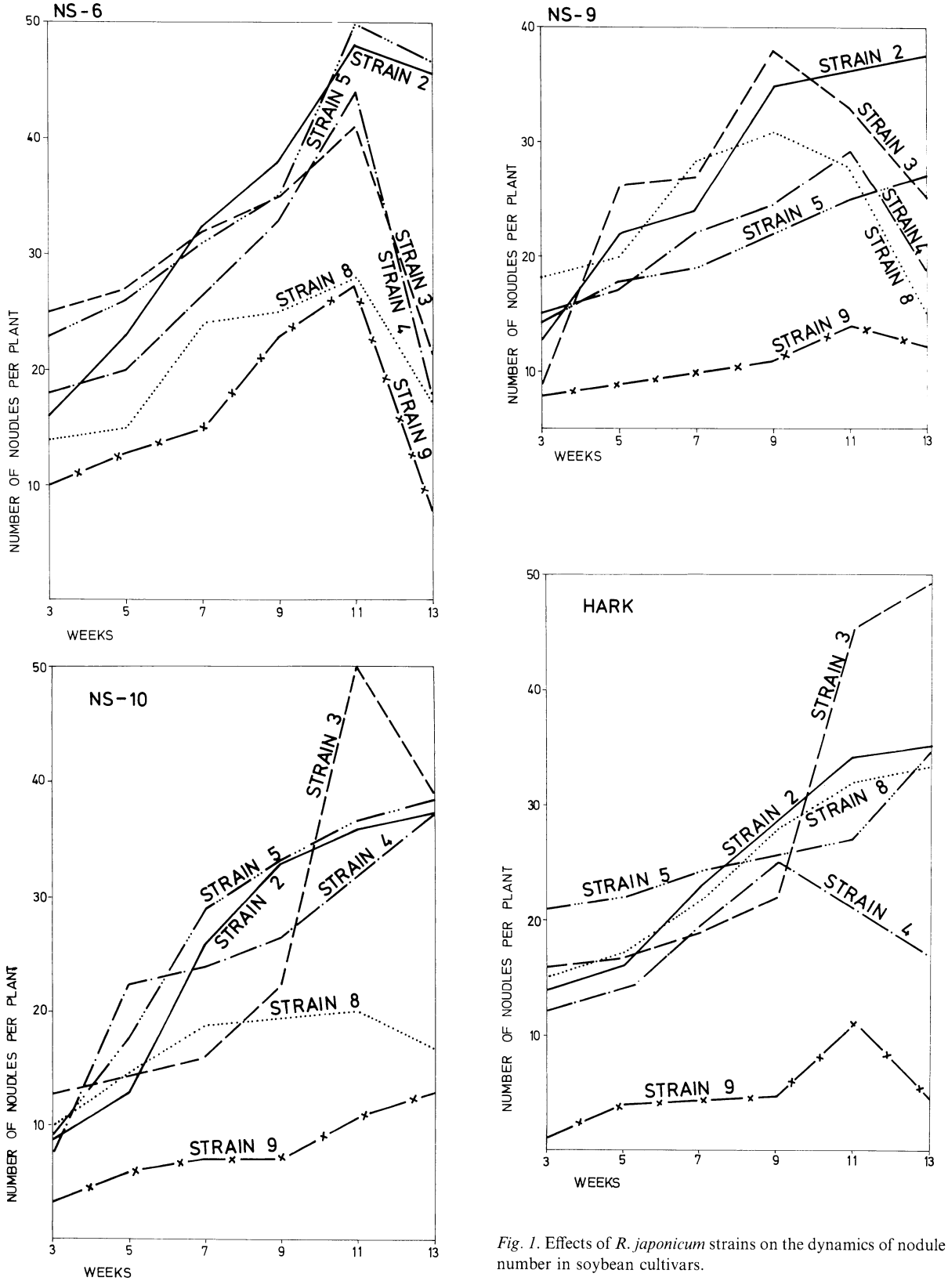

Fig. 1. Effects of *R. japonicum* strains on the dynamics of nodule number in soybean cultivars.

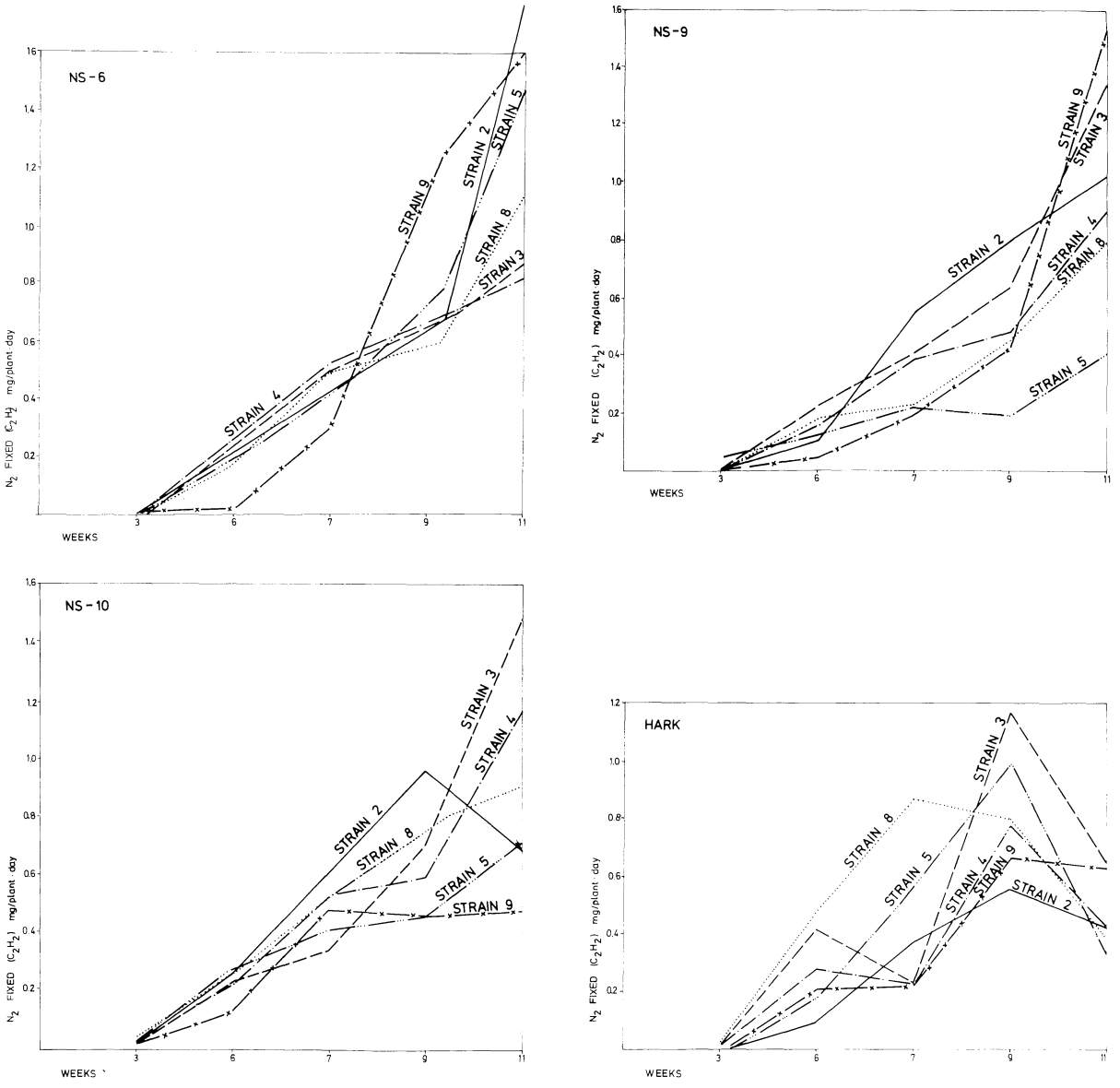

Fig. 2. Effect of *R. japonicum* strains on the dynamic of nitrogenase activity.

Cultivar NS-6

The number of nodules varied after 3 weeks from inoculation. The largest number of nodules was obtained with strain 2 and strain 5, and the least with strain 9. In this cultivar a number of nodules increase up to the 11th week and decreases after that period (Fig. 1). The nitrogenase activity also increased in the course of the season but certain differences were achieved depending on the strain.

With strain 9 the nitrogenase activity begins after 6 weeks while all the other strains begin earlier. The largest differences in the activity of nitrogenase were obtained after 9 weeks. In this period, strains 3 and 4 showed the lowest activity, while strains 9, 5 and 2 showed exceptionally high activity (Fig. 2).

The dynamics of nitrogen fixation by plants of this cultivar differed to a certain extent from the dynamics of nitrogenase activity, specially in the initial period. Although the activity of nitrogenase

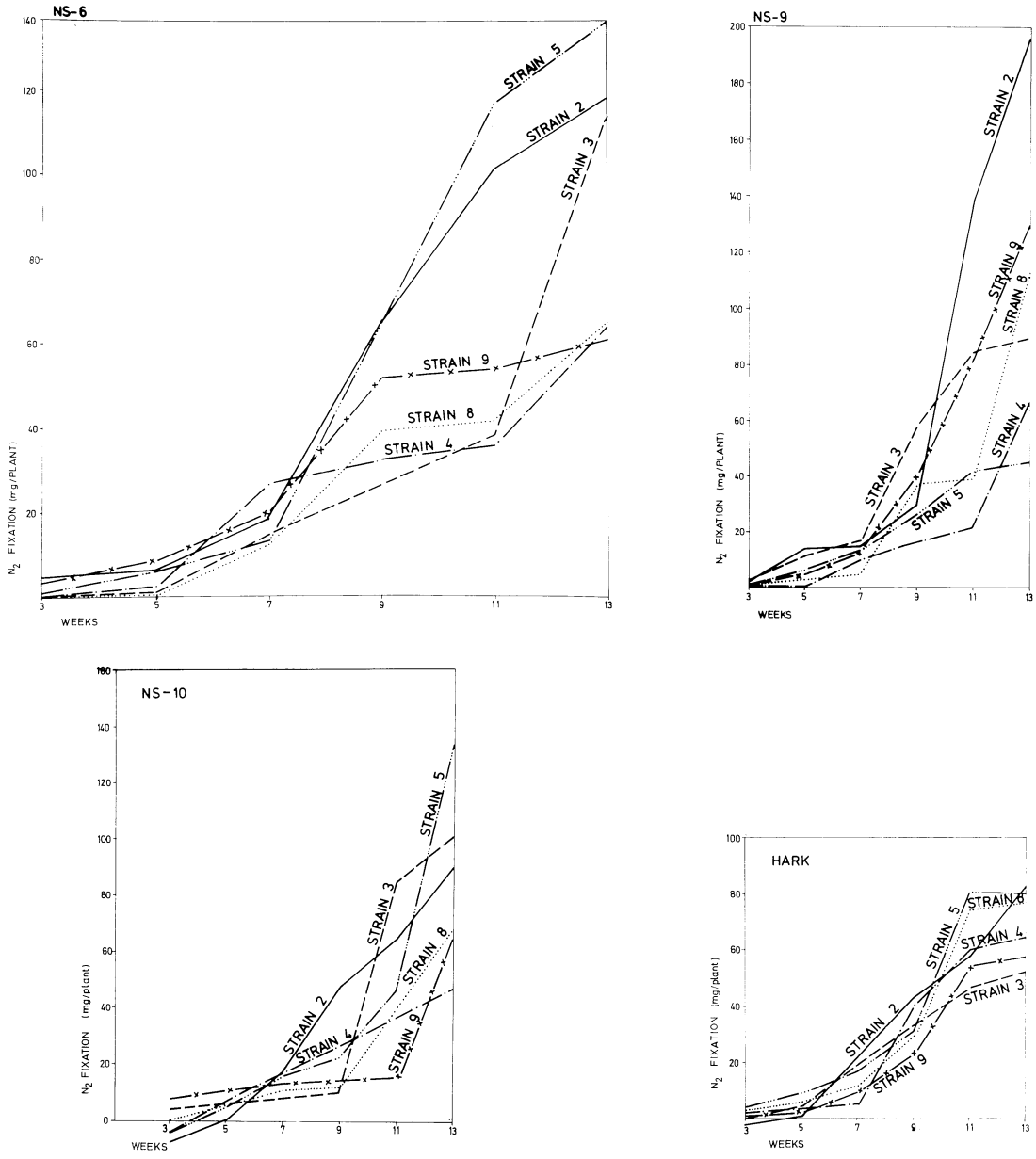

Fig. 3.. Effects of *R. japonicum* strains on the dynamics of nitrogen fixation in soybean cultivars.

in this period was detected in a number of strains, the quantity of fixed nitrogen was negligible. The largest quantity of fixed nitrogen was obtained with strains 5 and 2, and the least with strains 4 and 3 after 11 weeks of planting, the latter completely corresponding to the activity of nitrogenase. However, the nitrogen content in plants also increased after 11 weeks. Only with strain 9 the correlation between quantity of fixed nitrogen and nitrogenase activity was not obtained. The sudden decrease in the number of nodules after 9 weeks apparently influenced the decrease in quantities of fixed nitrogen from strain 9. (Fig. 3).

Cultivar NS-9

The number of nodules is somewhat lower than

in NS-6. However, the number varied even with this cultivar with different strains of *R. japonicum*. The lowest number of nodules during vegetation was obtained with strain 9 and the largest with strain 3. The largest number of nodules was obtained within the period of 9 to 11 weeks (Fig. 1).

Nitrogenase showed some activity after 3 weeks in all strains. However, differences in dynamics were evident, strain 5 showing the least increase and strains 9 and 3 the largest (Fig. 2). The quantity of fixed nitrogen for cultivar NS-9 also shows specific dynamics. The smallest quantity was fixed with strains 5 and 4, and largest with strains 2 and 9.

The dynamics of strain 2 is interesting showing that the quantity of fixed nitrogen depends not only on the activity of nitrogenase but also on the number of nodules in the period covering the 11th to 13th weeks. It should also be pointed out that the largest quantity of nitrogen was fixed by this cultivar (Fig. 3).

Cultivar NS-10

This cultivar is very similar to cultivar NS-9, with respect to the number of nodules at the beginning of growth. The least number of nodules was obtained with strain 9, the largest with strain 3. However, strain 5 caused a successive increase in the number of nodules in the entire tested period (Fig. 1).

In the activity of nitrogenase certain correlation between number of nodules and activity of nitrogenase with strains 9, 8 and 4, as well as, with strain 3 was found, with which was also obtained the largest activity, but with 11 week plants (Fig. 2).

The dynamics of the quantity of fixed nitrogen shows the largest differences between the strains after the 11th and 13th week. The largest quantity of fixed nitrogen was obtained with strain 5 which had the largest number of nodules after 7 weeks, but did not show a high nitrogenase activity. A certain correlation in the activity of nitrogenase and quantity of fixed nitrogen up to 11 weeks was obtained with strain 3 (Fig. 3).

Cultivar Hark

Different numbers of nodules were obtained with the tested strains already after 3 weeks. The lowest number of nodules was obtained with strain 9 and the largest with strain 3, the same as with variety NS-10 (Fig. 1). Six weeks after the inoculation, a very high activity of nitrogenase was obtained with all strains, especially with strains 8 and 3. However, the activity of nitrogenase with strains 3, 4 and 9 decreases after 7 weeks, to reach a maximum with strains 3 and 5 in the subsequent period. After 11 weeks, the activity of nitrogenase again decreases with all the strains (Fig. 2). The lowest quantity of nitrogen was fixed after 13 weeks by strain 3 and then strain 9. The largest quantity of fixed nitrogen was obtained with strains 2, 5, and 8. However, this cultivar fixed the least quantities of nitrogen in comparison with the other tested cultivars. (Fig. 3).

Discussion

It may be concluded that the quantity of nitrogen fixed in the course of 13 weeks depends both on the soybean cultivar and *R. japonicum* strain. These results further confirm the results obtained previously (Sarić *et al.*, 1987; Sloger, 1969).

Nodulation, nitrogenase activity and the content of fixed nitrogen may be used in the determination of symbiotic efficacy of soybean genotypes and bacterial strains.

The differences in nitrogenase activity among different strains have already been obtained after 7 weeks of growth. However, in the following period, the intensity of nitrogenase activity may change and it depends on the soybean cultivar and strain. *R. japonicum* strains in which nitrogenase activity increased continuously till the end of the period most often fixed much higher quantities of nitrogen. Thus, the nitrogenase activity of all strains of *R. japonicum* in the cultivars NS-9 and NS-6 increased to the end of the period and thus these two cultivars fixed even higher quantities of nitrogen. The highest nitrogen fixation was observed with *R. japonicum* strains 2 and 5. We may conclude that the cultivars NS-9 and NS-6 had the most effective system of nitrogen fixation with *R. japonicum* strains 2 and 5.

If the activity of nitrogenase varies, whether it decreases earlier or its intensity decreased in the course of the experiment as in Hark, it leads to the decrease of the content of the fixed nitrogen.

The number of nodules was more or less similar for all cultivars and mostly it characterized the

particular strain of *R. japonicum*. Thus, strain 9 rendered the lowest number of nodules and strain 3 the highest. The highest number of nodules is formed up to the eleven weeks of growth. In the successful systems (NS-9, NS-6), the number of nodules usually decreases after the end of this period.

The best correlation of the dynamics of nitrogenase and the content of the fixed nitrogen is observed in the cultivars NS-9 and NS-6, which justifies us in supposing that, in these cultivars, the process of nitrogen fixation is best correlated with other physiological processes. On the other hand, in Hark, the correlation of nitrogen fixation with all strains is lowest, so the lowest content of fixed nitrogen is obtained. We consider that in these cultivars, the characteristic capacity to use atmospheric nitrogen in nutrition is genetically predetermined and that specific nitrogen fixation is also characteristic of *R. japonicum* strains. These results prove our hypothesis that the functioning of the symbiotic system cultivar-strain depends on the correlation of both parties.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 391–395.
PLSO PN 180
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Differences between cereal crop cultivars in root-associated nitrogen fixation, possible causes of variable yield response to seed inoculation

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Key words: cereal cultivars, nitrogen fixation, phytohormones, rhizosphere bacteria, root pathogens, seed inoculation, yield response

Abstract

Large differences in N_2-ase activity with fractions of active plants from 3–67% and maximal activities from 3–35 nmol $C_2H_4.h^{-1}$ were found between sterile, Azospirillum-inoculated seedlings of 14 German cereal cultivars. Examples of similar cultivar differences in gnotobiotic or unsterile cereals in response to Azospirillum inoculation, in root exudation and the specificity of bacteria–root interactions are reviewed. As possible causes of yield responses to bacterial seed inoculation N_2-fixation, plant growth regulating metabolites and bacterial interaction with root pathogens are discussed. The need for suitable screening methods to select and breed cultivars with desirable responses to beneficial rhizosphere bacteria is pointed out.

Differences in associative nitrogen fixation between cereal cultivars

Screening and breeding experiments using sterile-grown, inoculated cultivars

Nine wheat-, four barley and a rye cultivar seedlings were sterile grown in semisolid mineral agar without N, inoculated with *Azospirillum brasilense* or *A. lipoferum* and assayed by the C_2H_2 reduction test for 24 h. These cultivars showed large differences with respect to their fractions of active and to their maximal nitrogenase (N_2-ase) activity, the eight summer wheat cultivars being most different with 3–68% active and 3–38% plants with higher activity, respectively. Three summer wheat and all three summer barley cultivars had 50% or more seedlings with N_2-ase activity (Table 1). Activities within cultivars generally followed a log-normal distribution (Jagnow, 1985). During unsterile pot cultivation of seedlings from summer wheat cv. 'Max' and summer barley cv. 'Koral' in the greenhouse, active and inactive plants did not show many differences until maturity. However, ear emergence took place two days earlier, shoot dry weights and N contents were lower, but root dry weights and 1000 grain weights were higher with active plants from both summer cereals. When F 1-seedlings from self-pollinated ears were tested for N_2-ase activity, seedling sets from ears of both active and inactive plants could contain no, few or larger numbers of active plants, the overall progeny from active and inactive plants being statistically not different (Jagnow, 1987).

Similar studies with inbred lines of *Pennisetum americanum* showed that all inoculated plants were positive with respect to the 'nis' character (*ni*trogen fixation *s*upporting) and distinct, different levels of N_2-ase activity were found between lines within the range between 0.2 and 23 nmol $C_2H_4\,h^{-1}$. The F 1 generation generally has 'nis' levels as the parent plants (Bouton and Brooks, 1982; Bouton *et al.*, 1985). Breeding analysis of *P. typhoides* suggested that both dominant and recessive genes were responsible for 'nis' expression (Manga *et al.*, 1985). Since the German cereal cultivars represented no genetically defined lines and were never selected for the 'nis' character, different fractions and a con-

Table 1. Frequency and rate of nitrogenase activity of sterile seedlings of German cereal cultivars after incoulation with *Azospirillum brasilense* or *A. lipoferum*

	Number of plants	% of active plants		Maximal N_2 ase-activity (nmol $C_2H_4\,h^{-1}$)
		$>0.2\,nmol\,h^{-1}$	$>5\,nmol\,h^{-1}$	
Summer wheat				
"Max"	63+	68^{++}	38_a	34.2
"Amor"	60	57_{ab}	23_{ab}	21.1
"Turbo"	60	50_{ab}	17_{bc}	12.4
"Kokat"	50	48_b	14_{bc}	12.0
"Achill"	12	42_{bc}	25_{ab}	42.2
"Horizont"	60	37_{bc}	5_{bc}	13.3
"Sokrates"	47	36_{bc}	6_{bc}	11.0
"Kadett"	30+	3_d	3_{bc}	7.3
Winter wheat				
"Disponent"	51+	25_c	6_{bc}	13.5
Summer barley				
"Golda"	62	63_{ab}	27_{ab}	33.6
"Koral"	55	60_{ab}	16_{bc}	20.8
"Georgi"	62	56_{ab}	26_{ab}	35.4
Winter barley				
"Diana"	67	46_b	10_b	10.6
Summer rye				
"Sorom"	62	24_{bc}	3_c	5.6

+ inoculated with *A. lipoferm.*

++ values with different index letters are significantly different ($P = 0.05$ or less) according the chi-square test.

tinuum from low to high activities were found. Therefore, this selection was only a first step to genetic segregation and a heterogeneous expression of the 'nis' character should be expected in the F 1 generation. Nevertheless, different fractions of active plants and different ranges of activities seem to be characteristics of distinct cultivars.

Cultivar differences in associative nitrogen fixation in unsterile pot and field experiments

Large differences in N_2-ase activity of root-soil cores or excised roots were detected between field-grown maize hybrid and inbred lines in New Jersey by De-Polli *et al.* (1982). Plants were not inoculated and close association of indigenous dazotrophs with the plants was inferred from corresponding variations of activity with day-night cycles. Similar differences were reported by Rennie and Thomas (1987) between wheat varieties in field trials in Canada, inoculated with *Azospirillum brasilense* or *Bacillus polymyxa* by using the ^{15}N isotope dilution method. Fixation rates of up to $52\,kg\,N\,ha^{-1}$ were calculated for cv. 'Cadet', whereas cv. 'Rescue' ranged at the lower end of eight varieties tested. Cultivar × variety interactions suggested the need for using bacterial strains adapted to particular varieties. Also there was little correlation between % of plant-N fixed and yields, the plants using less soil-N correspondingly. Spontaneous differences in N_2-fixation between rice cultivars during flooded cultivation by indigenous, enriched rhizosphere bacteria were reviewed by Watanabe (1984). These were detected by conventional Kjeldahl and N-balance methods. He also stressed the need for simple and rapid screening methods to breed varieties high in N_2-fixation.

Different yield responses of cultivars to seed inoculation

Even without N_2-fixation sufficient to account for yield responses, many cereal cultivars tested showed different responses to inoculation with

Azospirillum spp. For instance, from 42 Israeli wheat cultivars inoculated with a mixture of five *A. brasilense* strains in a pot trial, only 6 showed significantly positive grain yield responses of 42–126% (Avivi and Feldman, 1982). In more recent field inoculation trials with wheat, less spectacular cultivar dependent positive yield responses to *A. brasilense* (0–26%) were observed in Israel (Kapulnik *et al.*, 1983; Kapulnik *et al.*, 1987; Millet *et al.*, 1985), in Canada (Jain and Patriquin, 1984) and in Belgium (Reynders and Vlassak, 1982). Similarly divergent yield responses of cultivars were also obtained with *Sorghum* spp. and/or *Pennisetum* spp. in Florida (Smith *et al.*, 1984), in Egypt (Eid *et al.*, 1984) and in India (Wani *et al.*, 1985). For a more detailed review see Jagnow (1987a).

Possible causes of variable yield responses

Fixed nitrogen and cereal yield

Recent pot and field experiments show that N_2-fixation of inoculated Azospirillum in the rhizosphere of cereals is very low. For instance, in pot experiments of Lethbridge and Davidson (1983) with wheat, using the ^{15}N isotope dilution method it was not detectable. In another pot experiment with pearl millet, wheat and maize O'Hara *et al.* (1987) compared inoculation treatments with N_2-fixing strains and a nif (−) mutant strain of *A. brasilense*. They found no significant yield differences with the nif (−) strain and low and very variable rates of N_2-ase (C_2H_2) activity on excised roots. Similar measurements with wheat and barley roots from a field experiment with different N-fertilizer doses in Germany also showed very low N_2-ase activities by indigenous rhizosphere bacteria (Jagnow, 1983). ^{15}N-dilution and C_2H_2-reduction measurements with inoculated, field-grown wheat in Israel showed only low and transient N_2-ase activities at earlier growth stages and no N_2-fixation based on ^{15}N-dilution (Kapulnik *et al.*, 1985). Under similar conditions in Israel Bashan (1986) reported that inoculated Azospirillum reached very low rhizosphere populations already after three weeks. These results show that the contribution of N_2-fixation to the N-uptake of cereals is negligible and may be estimated as 1 kg ha^{-1} or less.

Bacterial excretion of growth regulating substances

Cereal plants inoculated with *A. brasilense* produce more tillers, side roots and root hairs if grown under sterile or unsterile conditions (Okon, 1984). Like many other growth-promoting rhizosphere bacteria, Azospirilla produce indole acetic acid (IAA) also in media without tryptophane during the early stationary growth phase (Horemans and Vlassak, 1985). Inoculation of plants with an IAA overproducing mutant increased numbers and total length of roots and root hairs as did application of 10^{-9} g/L IAA solution to roots of sterile grown plants (Kolb and Martin, 1985). *Azospirillum brasilense* may also excrete a gibberelline stimulating side root, hair and mucigel formation with ensuing heavy population of root tips (Tien *et al.*, 1979; Umali-Garcia *et al.*, 1980). *In vivo* phytohormonal activity was also shown with IAA deficient dwarf mutants of wheat, being restored to normal growth by Azospirillum inoculation in unsterile soils (Inbal and Feldman, 1982). A specific reaction of root hairs of wheat to *A. brasilense* analogous to legume root hairs after contact with Rhizobium consisted of widenings and 'tune-fork-like' branchings with wheat root isolates only (Jain and Patriquin, 1984). Bacterial production of such substances during growth on root surfaces, however, has not been demonstrated yet. Therefore, bacterial stimulation of plants to increased synthesis of growth regulators may also be possible.

Bacterial influence on root pathogens and symbionts

Some rhizosphere bacteria, mainly fluorescent pseudomonads, are able to promote root growth by successful competition with fungi and bacteria inhibitory or even pathogenic to roots. Many strains of widely distributed soil bacteria such as *Pseudomonas fluorescens*, *P. putida*, Enterobacter, Klebsiella, Flavobacterium and Arthrobacter produce metabolites toxic to roots. Only 3–5% of

the fluorescent pseudomonads present could enhance root growth by competing and reducing the rhizosphere populations of such organisms (Iswandi *et al.*, 1987; Suslow and Schroth, 1982). Success of inoculation in such cases depends on the size of populations of root-deleterious bacteria and fungi in field soil. The ability to displace such organisms from the rhizosphere may depend on the formation of antibiotic substances and/or iron chelating siderophores (Kloepper *et al.*, 1980; Kloepper and Schroth, 1981), but growth promotion by growth-promoting pseudomonads, possibly via auxin production, was also demonstrated in the absence of root deleterious organism (Elad *et al.*, 1987). Whether *Azospirillum* spp. or other N_2-fixing, growth promoting rhizosphere bacteria may also act by displacements of root pathogens, is not known at present.

Positive effects of rhizosphere bacteria may also result from enhancement of root infection by symbiotic VA-mycorrhiza fungi. Inoculation of maize and ryegrass with *A. brasilense* and/or *Glomus mossae* resulted in increased root infection by Glomus and increased P-uptake if both organisms were inoculated simultaneously (Barea *et al.*, 1983). Similar results were obtained by Subba Rao *et al.* (1985) with *Pennisetum americanum* and by Pacovsky *et al.* (1985) with *Sorghum bicolor*, using different soil types. The latter showed, that enhancements of mycorrhiza infection by *A. brasilense* were stronger in soils with a high capacity of P-fixation. Enhanced infection and P-uptake of *Paspalum notatum* and *Panicum virgatum* with *Glomus macrocarpus* by simultaneous inoculation with *A. brasilense* was also pH-dependent (Menze, 1985). The pH of the low nutrient experimental soil was raised from 4.5 to 5.5 and 6.5 by liming. Positive yield effects were observed only in soil with pH 6.5, although increased infection by the fungus took place also at the lower pH levels. Some authors (*e.g.* Dehne, 1986) report that VAM-infection by itself can reduce infection of cereals by root pathogenic fungi. It seems likely, that this protective effect may also be enhanced by inoculation with bacteria, which increase infection.

Conclusion

The examples given show that genotypic variability exists between and within cereal cultivars in their ability to support nitrogenase activity of root-associated bacteria. The expression of the 'nis' character would require the excretion of sufficient organic compounds with an overall N-content low enough not to repress nitrogenase synthesis and function of such bacteria. Whether nis-positive cultivars are also enhancing root colonization of rhizosphere bacteria stimulating plant development by growth regulating substances and/or protection from harmful microorganisms and are benefitting more from it than nis-negative cultivars remains to be shown. The ability to promote a protective rhizosphere microflora is likely to depend on the excretion of low-N root exudates also in the presence of $N_{min.}$-fertilizers ensuring near maximum yields. An increase of phytotoxic hyphomycetes has been reported by Zvyagintsev and Gutsev (1987) to occur with optimal to superoptimal doses of $N_{min.}$-fertilizers. Possibly also rhizosphere bacteria detrimental to root growth may be favoured by high or excessive doses of $N_{min.}$-fertilizers. Clearly, also other rapid screening tests have to be developed for breeders to select cultivars able to benefit most from inoculation with growth promoting, root protecting rhizosphere bacteria and symbiotic VAM fungi.

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PLSO PN 181
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Genetical analysis of the efficiency of VA mycorrhiza with spring wheat

I. Genotypical differences and a reciprocal cross between an efficient and non-efficient variety

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Key words: genetical analysis, *Glomus manihotis*, High Yielding Varieties, Landraces, P deficiency, plant height, reciprocal crosses, root length, *Triticum* spp, VA mycorrhizal efficiency, wheat

Abstract

To quantify the variability of the mycorrhizal efficiency in wheat, wild forms, Landraces (LRs), and High Yielding Varieties (HYVs) were investigated with respect to dry weight of the ears and shoots, plant height, total root length, P concentration, P uptake, infection rate of the VAM fungus *Glomus manihotis* in wheat roots and total infected root length

Under P deficiency the VA mycorrhiza showed a higher efficiency in growth response with most of the LRs than with HYVs. The efficient wild variety *Triticum aestivum rotundatum* and the African HYV 'Huanca' were crossed reciprocally. Chromosomal heredity could be observed. In some genotype x environment interactions differences between the reciprocal F_1 indicated also plasmatic effects.

Introduction

VA mycorrhiza (VAM) can improve the P uptake from hardly soluble, soil-borne or applied P compounds. This is particularly relevant for plant production on soils with a high P fertilizer fixation, such as the acid latosoles prevalent in the humid tropics with high Al and Fe contents and the alkaline, calcareous soils of the subtropics (Moawad, 1979). Screening experiments with different plant species showed a high genotypical variability in VA mycorrhizal infection and efficiency: *Triticum aestivum* (Bertheau *et al.*, 1979), *Trifolium repens* (Crush and Caradus, 1980), *Pisum sativum* (Jensen, 1985), *Solanum tuberosum* (Bhattari and Mishra, 1984), *Arachis hypogaea* (Krishna and Williams, 1987), *Pennisetum glaucum* (Krishna and Lee, 1987), *Medicago sativa* (Lackie *et al.*, 1988) and various other species (Graw *et al.*, 1979). Often varieties either slightly or not improved by breeding showed higher yield increases by inoculation with VA mycorrhizal fungi than so-called High Yielding Varieties (HYVs), indicating, that the VA mycorrhizal efficiency depends not only on environmental conditions and efficiency of the VA mycorrhizal fungus but also on the genotype of the host plant. This is important for the breeding of low-input cultivars with little need of fertilizer, especially in combination with inoculation of effective strains in field soils.

In this work the inheritance modus of the VA mycorrhizal efficiency in spring wheat has been investigated. In the first step the variability of the VA mycorrhizal efficiency is quantified, especially between Landraces and wild-forms on one hand and HYVs on the other hand at two P levels.

Materials and methods

Experimental design

The wheat plants were grown in plastic pots of 15 cm diameter with 2 kg soil under glasshouse con-

ditions under natural sunlight. The soil substrate was composed of a P-deficient and P-fixing, sandy forest soil from Ebergötzen, near Göttingen, FRG, with high contents of Al, Mn and Fe, mixed with quartz sand in the proportion of 1:1. The soil was watered to field capacity and limed with $CaCO_3$ to pH 5.5. This pH has proved best for the VA mycorrhizal fungus used, *Glomus manihotis*, a very efficient strain.

Before application of the P forms the soil substrate was sterilized at 120 °C for 6 h. Hardly soluble iron phosphate ($FePO_4$) and well soluble monocalcium phosphate (MCP) was carefully mixed with the sterilized air-dried soil, 30 mg P kg^{-1} dry soil corresponding, to 90 kg P ha^{-1} (calculated for 20 cm depth and 1,5 g cm^{-3} soil). Basic nutrients except P were applied in solution after germination or transplanting.

VA mycorrhizal inoculum consisted of root pieces of *Eupatorium odoratum* infected with *Glomus manihotis*. For the screening experiment the seeds were sown directly into the pots. For the comparison of the P and F_1 generations, wheat seeds were germinated in dishes and kept for 4 weeks under controlled low temperature of 5 °C, to stimulate tillering. The four-week-old plants were transplanted into the experimental pots.

The screening experiment was conducted in the summer of 1985. Twenty-two High Yielding wheat Varieties from German plant breeders and 22 Landraces or wild forms, randomly selected from gene banks, were investigated.

In the winter of 1985/86, the Landrace *Triticum aestivum rotundatum* (a VAM efficient variety) and the African HYV 'Huanca' (non-efficient) were reciprocally crossed to obtain seeds for the comparison of the P and F_1 generations in summer 1986. The self-pollinating wheat flowers were emasculated and pollinated with father ears under cellophane bags.

Experimental evaluation

The VA mycorrhizal efficiency is defined as the degree to which a genotype yields better with than without VAM in a deficient soil.

The plants were harvested at milk-ripe stage of the grains. Roots were washed and separated from soil and root fresh weight was obtained. For the determination of root length representative samples of the roots were collected. Root length was measured by the gridline intersect method (Giovanetti and Mosse, 1980). Representative root samples were fixed in 50 % alcohol and stained as described by Phillips and Hayman (1970). The infection rate was assessed according to the subjective estimation method in breeding programms for mildew resistance in wheat. The infection rate represents the proportion of root length infected by VA mycorrhizal fungus. The total infected root length was calculated by multiplying infection rate with root length. Plant height was obtained by the distance from soil level to the top of the ear. Shoots were dried at 80 °C for 3 days, separated into ears and straw and their dry weight were determined. The shoot yields consisted of the ear plus straw dry weights. P concentrations in the shoots were measured photometrically by the vanadate-yellow method (Kurmies, 1955). P uptake was calculated by multiplying P concentration with dry weight.

Both experiments had a 3-factorial design, with P treatments, VAM treatments and genotypes. Treatment means were compared by using an analysis of variance (ANOVA). When F ratio was significant, differences were statistically evaluated by the TUCKEY multiple range for $P = 0.05$.

Results

The $FePO_4$ treatment represents an extreme P deficiency so that the wheat plants remained small and did not tiller. Fertilized with soluble MCP, the wheat plants grew much better. Because of the great differences between $FePO_4$ and MCP fertilized plants, the P treatments had to be calculated statistically separately. In the first experiment, 44 wheat varieties were nested within two groups—*i.e.* High Yielding Varieties (HYVs) and Landraces (LRs). In the second experiment the genotypes consisted of two parental and their reciprocal F_1 generations. In all mentioned parameters of both experiments VAM treatment × wheat genotypes interactions were significant.

The screening

With $FePO_4$ the wheat roots were much more infected than with MCP (Table 2).

Table 1. Differences between 22 High Yielding Varieties (HYVs) and 22 Landraces and wild forms (LRs) of spring wheat in several parameters, non-inoculated (NM) and inoculated (M) with the VA mycorrhizal fungus *Glomus manihotis*, fertilized with $FePO_4$ (a) or monocalcium phosphate (b, MCP), multiple range test: TUCKEY (a = ab, ab = b, a < b < c *etc.*)

a) *fertilized with $FePO_4$*

	Ear dry weight (mg/pot)			Shoot dry weight (mg/pot)			Plant height (cm)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
LRs	350 a	504 d	148*	1023 a	1260 b	113*	50 b	53 b	105
HYVs	384 ab	420 c	114*	1026 a	1053 a	99	42 a	40 a	96
	Shoot P concentration (mg P/g dry weight			Shoot P uptake (mg P/pot)			Root length (m/pot)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
LRs	3.3 a	4.9 a	151*	3.4 a	6.2 c	188*	52 b	57 b	112
HYVs	3.2 a	4.2 b	134*	3.2 a	4.3 b	139*	38 a	40 a	107

b) *fertilized with MCP*

	Ear dry weight (mg/pot)			Shoot dry weight (mg/pot)			Plant height (cm)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
LRs	1774 c	1570 b	89*	3538 c	3081 b	85*	70 d	65 c	92*
HYVs	2040 d	1306 a	65*	3617 c	2323 a	72*	56 b	51 a	90*
	Shoot P concentration (mgP/g dry weight			Shoot P uptake (mg P/pot)			Root length (m/pot)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
LRs	7.7 a	8.3 b	109*	26.6 bc	25.0 b	94	98 cd	82 bc	84
HYVs	8.0 ab	8.4 b	106	27.8 c	19.5 a	70*	65 b	47 a	74*

* significant differences between NM and M for LSD 5%

All 44 wheat varieties were infected by the VA mycorrhizal fungus *Glomus manihotis*. The influence of mycorrhizal inoculation on different phenotypical parameters of wheat are presented in Figure 1 and Tables 1 a,b and 2.

Table 2. Differences between 22 High Yielding Varieties (HYVs) and 22 Landraces (LRs) of spring wheat in infection rate (%) and total infected root length (m/pot), inoculated the VA mycorrhizal fungus *Glomus manihotis*, fertilized with $FePO_4$ or monocalcium phosphate (MCP), data of infection rate were transformed for statistical evaluation by arc sin λ%, multiple range test: TUCKEY (a = ab, ab = b, a < b < c)

	Infection rate (%)		Total infected root length (m/pot)	
	$FePO_4$	MCP	$FePO_4$	MCP
LRs	42 b	7.9 a	27.0 c	9.0 ab
HYVs	35 b	5.4 a	15.1 b	2.6 a

The effect of the symbiosis was different at low ($FePO_4$) and high P fertility conditions (MCP). The MCP-fertilized plants yielded 3 to 5 times higher dry weights than $FePO_4$-fertilized plants. The ears benefited more from the better P availability than the straw resulting in greater ear proportions. The yields responded more strongly than the root lengths. The high level of available P in the MCP treatments also raised P concentration and P uptake (Table 1 a,b).

P deficient level ($FePO_4$). Ear and shoot dry weight were increased by *G. manihotis* under P deficient conditions ($FePO_4$), especially with Landraces and wild forms of wheat. On the average, inoculated with *G. manihotis* the 22 LRs yielded more than the

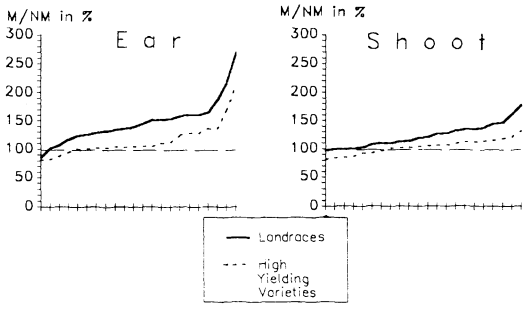

Fig. 1. VA mycorrhizal efficiency in ear and shoot yield (M/NM in %) of 22 High Yielding Varieties and 22 Landraces or wild forms of wheat, non-inoculated (**NM**) or inoculated (**M**) with *Glomus minihotis* and fertilized with $FePO_4$. – – –: 100 %, no effect by VAM.

22 HYVs, although without VA mycorrhizae there were no differences between the LRs and HYVs. The consequence is, that non-improved varieties (LRs) showed a higher VA mycorrhizal efficiency (M/NM in %) (Table 1a). The majority of the primitive wheats (LRs) ranged in their VA mycorrhizal efficiency of ear dry weight above 130 %, 20—30 % more than the mean of HYVs (Fig. 1). The wild form *Triticum vavilovii*, the Landraces 'Weender Galizischer Kolben' and *T. durum reichenbacchii*, but also the HYVs 'Bali' and 'Mexikana A1/9' doubled their ear dry weight with VAM. 'Weender Glizkolben', 'Roter Schlanstedter' and 'Schwarzer Spelzweizen', with the highest dry weight of all 44 genotypes increased the ear more than the shoot dry weight (Table 1a and Fig. 1), and only in LRs significant increases of the shoot dry weights with VAM could be found (Table 1a).

The mean plant height of the HYVs was shorter than that of the LRs. Plant height and root length were positively correlated (Table 1a). LRs produced greater root length than HYVs on the average (Table 1a). HYVs showed less variation in root length and plant height than LRs. There was no significant effect of VAM on plant height and root length (Table 1a). Only the LRs 'Weender Galizischer Kolben', 'Mahndorfer Burgunder' and 'Schwarzer Spelzweizen' grew higher, and the HYV 'Mexikana 104872' shorter with VAM.

The Landraces 'Roter Schlanstedter', *Triticum aestivum ferrugineum* and 'Spanisches Einkorn' with long haulms had very intense root systems, but the non-improved durum wheat 'Malta Yellow' demonstrates a variety with an intense root system and short halm.

There were no differences in the infection rate of *G. manihotis* between HYVs and LRs on the average (Table 2). But some primitive wheats like 'Weender Galizischer Kolben', 'Roter Schlanstedter', *T. ae. multurum compactoides* and 'Weißer Grannenspelz', and some HYVs like 'Bali', 'Bastion' and 'Nungu' were strongly infected (above 70 %). Corresponding with their intenser root systems the LRs showed greater infected root length than HYVs (Table 2). Especially the combination of many roots and high infection rate caused in the Landrace 'Roter Schlanstedter' 78 meter total infected root length, more than 3 times higher than the average. There are HYVs with short but strongly infected roots like 'Bastion' and 'Bali', on the other hand, however, LRs with a very intense but less infected root system like 'Chinese Spring', *T. spelta saharense, T. durum reichenbacchii* and *T. aestivum erythrospermum*.

The correlations (r = correlation coefficient) were weak between the VA mycorrhizal efficiency in shoot dry weight on one side and infection rate or total infected root length on the other. But with r = 0.22 and r = 0,31 for HYVs there is a tendency for a positive correlation between the efficiency of VAM and the total infected root length.

VAM promoted the P concentration and P uptake in shoots in LRs more than in HYVs (Table 1a). Without VAM there were no differences in the mean of P concentration between HYVs and LRs. The inoculation effect on the individual varieties differed strongly. In many varieties no inoculation effect on P concentration could be observed, but in the wild form *T. ae. vavilovii*, the Landrace *T. ae. multurum compactoides* and the two African HYVs 'Huanca' and 'Nungu' the P content was doubled by VAM. Because of the greater yield increase by VAM in Landraces and wild wheats their shoot P uptake exceeded that of the HYVs (Table 1).

Under P deficiency ($FePO_4$) an intense, but also well infected root system promoted the P uptake into wheat shoots. The inoculation effect (M/NM in %) on shoot P uptake was positively correlated with the infection rate (r = for the LRs 0,46 and HYVs 0,63) and the total infected root length (r = for LRs 0,43 and HYVs 0,57).

High P fertility level (MCP). The VAM fungus

Glomus manihotis infected the wheat roots only poorly, when plants were well fed with P due to MCP fertilization (Table 2), and VAM showed parasitic effects, with HYVs more than with LRs (Table 1b). VAM reduced the means of the ear and shoot dry weights more with the HYVs than with the LRs (Table 1b). Eighteen HYVs and six LRs showed significant parasitism in respect of shoot dry weight by VAM.

With MCP, just like $FePO_4$, the LRs showed greater plant height and root length, with and without VAM (Table 1b). Especially the HYVs reacted under M/MCP conditions with shorter haulms and smaller root systems (Table 1b). With LRs the parasitic effect on the root length was not significant, in contrast to their ear and shoot yield.

There was no reduction in P concentration with VAM inoculation. With LRs the P concentration was even slightly promoted by VAM. Because of the yield reducing effect of VAM inoculation the P uptake in shoots was reduced by VAM, significantly only with the HYVs.

Inheritance of the VA mycorrhizal efficiency in a reciprocal cross between an efficient and a non-efficient variety

The comparison between the parental and F_1 generations of the African HYV 'Huanca' (HU) and the Landrace *Triticum aestivum rotundatum* (ro) was conducted during the warm summer 1986 with high light intensities. Under these growth conditions *Glomus manihotis* increased the ear and shoot dry matters with $FePO_4$ fertilization as well as with MCP (Fig. 2), the latter in contrast to results of the screening experiment in summer 1985 with cloudy weather where the same fungus acted parasitically in both parental varieties.

Summer 1986. The Landrace ro grew badly without VAM. With MCP the shoots of ro yielded even less than the High Yielding Variety HU and both F_1s (Fig. 2). *T. ae. rotundatum* showed a strong positive yield response to the symbiosis, resulting in the highest VA mycorrhizal efficiency for all factor combinations. With low P fertility in the soil ($FePO_4$) mycorrhizal ro even outyielded HU in ear dry weight. In contrast to HU the shoot growth response of ro by VAM was significant. In the

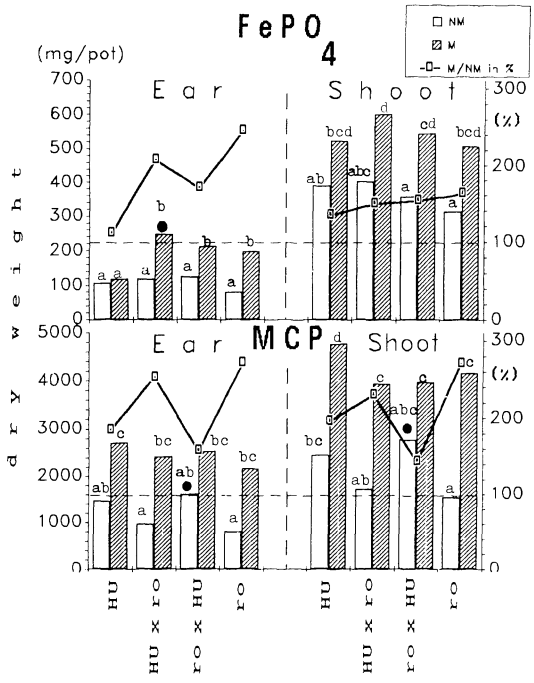

Fig. 2. Yield and VA mycorrhizal efficiency (M/NM in %) for ears and shoots of the P and F_1 generations of a reciprocal cross between the African High Yielding wheat Variety 'Huanca' (**HU**) and the Landrace *Triticum aestivum rotundatum* (**ro**), non-inoculated (**NM**) or inoculated (**M**) with the VA mycorrhizal fungus *Glomus manihotis* and fertilized with $FePO_4$ or monocalcium phosphate (**MCP**).
Columns: dry weight,
Curves: VA mycorrhizal efficiency, – – – line: 100 %, M = NM.
● significant differences between the reciprocal F_1, same VAM treatment, separately calculated, LSD 5 %.

MCP treatment the differences in shoot dry weight between ro and HU became considerably smaller (Fig. 2). The mycorrhizal efficiency (M/NM in %) was much greater with ro than with HU in respect of other characters.

The high VA mycorrhizal efficiency (M/NM in %) of the wild wheat in ear and shoot dry matter with the $FePO_4$ treatment could be transferred by reciprocal crossing to the non-efficient HU (Fig. 2). The ear and shoot dry weights of inoculated ro and F_1 plants were significant higher than without VAM. This was not true for HU. The efficiency of the F_1 ranged between that of the parents, but the absolute ear dry weight values even surpassed those of HU.

The effect of the inoculation was different in the MCP treatments. With VAM the shoots of HU

grew better than the other three genotypes, but with no differences in ear dry weights. With MCP only in the case of HU × ro the M/NM in % values of ear and shoot dry weight lie between the low efficiency of HU and the high of ro (Fig. 2, MCP). Statistically calculated alone, without the influence of the other factor combinations, the difference between the non-inoculated reciprocal F_1 was significant. The non-inoculated ro × HU— plants grew better than HU × ro, with the consequence that the relative yield increase by VAM was low (Fig. 2). Root length was increased by VAM, in the MCP treatment with all four genotypes, fertilized with $FePO_4$ only with HU and ro × HU (Table 3a). Independent from VAM treatment differences among the P and F_1 plants occurred only in the inoculated $FePO_4$ treatments: the root length of ro × HU were significantly greater than the reciprocal F_1 and ro.

Separately calculated, there was another difference in root length between both reciprocal F_1: with MCP non-inoculated ro × HU surpassed HU × ro (Table 3b). This corresponded with the better shoot growth of this hybrid (Fig. 2).

ro × HU showed also higher P concentration than HU × ro in the uninoculated $FePO_4$ treatment; ro × HU was equal to ro in this respect, HU and HU × ro were lower than the other two genotypes. The differences among the 4 genotypes in the VAM—$FePO_4$ treatment were not significant. Total P uptake was dominated by the growth pattern of the 4 genotypes; differences between the two hybrids were insignificant and lie between the two parents (Table 3a).

In the MCP treatments the P concentration and P uptake of mycorrhizal HU was superior to the other three inoculated genotypes. Non-inoculated, the P uptake of the F_1 ranged between both parents (Table 3b).

Discussion

Plant breeders can find many interesting properties in wild relatives of cultivated plants (Zohari, 1970). In this work it became evident that some wild wheats possess high VAM mycorrhizal efficiencies with better grain yields of the inoculated

Table 3. Effect of inoculation with the VA mycorrhizal fungus *Glomus manihotis* on parental and F_1 generations of reciprocal crosses between the African High Yielding wheat Variety 'Huanca' (HU) and the Landrace *Triticum aestivum rotundatum* (ro), non-inoculated (NM) or inoculated (M), fertilized with $FePO_4$ a) or monocalcium phosphate (b, MCP), multiple range test: TUCKEY (a = ab, ab = b, a < b < c *etc.*)

a)*fertilized with $FePO_4$*

	Root length (m/pot)			Shoot P concentration (mg P/g dry weight)			Shoot P uptake (10^{-3} mg P/pot)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
HU	21 a	31 bc	150*	0.35 a	0.62 bc	176*	140 a	328 abc	235
HU × ro	17 a	24 ab	146	0.59 ab	0.68 bc	117	236 ab	408 bc	173
ro × HU	16 a	●35 c	225*	●0.71 bc	0.79 bc	101	286 abc	437 c	152
ro	16 a	22 ab	139	0.74 bc	0.86 c	117	232 ab	442 c	190*

b)*fertilized with MCP*

	Root length (m/pot)			Shoot P concentration (mg P/g dry weight)			Shoot P uptake (10^{-3} mg P/pot)		
	NM	M	M/NM in %	NM	M	M/NM in %	NM	M	M/NM in %
HU	31 a	82 b	268*	2.33 ab	2.51 b	107	5.7 bc	11.9 b	205*
HU × ro	26 a	91 b	345*	1.99 ab	1.72 a	86	3.5 ab	6.7 c	191*
ro × HU	●37 a	84 b	226*	2.05 ab	1.74 a	85	5.3 abc	6.8 c	124
ro	32 a	99 b	315*	2.01 ab	1.67 a	83	3.1 a	6.9 c	222*

* significant differences between NM und M for LSD 5%.

● significant differences between the reciprocal F_1, same VAM treatment, separately calculated, LSD 5%.

plants, and this property can be transferred to a nonefficient HYV. In the screening, 44 spring wheat varieties showed differences in VA mycorrhizal efficiency, pointing to the probability that the benefit from VAM is also controlled by the genotype of the host plant. Under P deficient conditions ($FePO_4$), on the average 22 Landraces and wild forms showed higher VA mycorrhizal efficiency and yield than 22 High Yielding Varieties. In the reciprocal cross, the VA mycorrhizal efficiency could be transferred from an efficient wild wheat to a non-efficient HYV. Primarily, chromosomal genes were responsible for the mycorrhizal action in the wheat host plant, but in some genotype × environment interactions plasmatic gene effects could be observed.

Research on the genetic control of root-microorganism interactions, especially with mycorrhizal fungi, is still rare (Jensen, 1985; Krishna and Williams, 1987; Krishna *et al.*, 1985). When selecting for horizontal susceptibility to VA mycorrhizal fungi in *Pennisetum glaucum*, Krishna *et al.* (1985) found that genetic factors responsible for high levels of infection were "dominant" in the more susceptible genotypes.

The beneficial effect of vesicular-arbuscular mycorrhiza (VAM) is of special importance for those plants having a coarse and poorly branched root system. The external hyphae can absorb nutrients, especially with low mobility in soil solution like P, Zn and Cu, from a much larger soil volume than the absorbing zone surrounding a non-mycorrhizal root (Howeler *et al.*, 1987). The monocotylous wheat with a fine branched intense root system has an excellent capacity for nutrient uptake from the soil and does not depend so much on VAM as many other plants (Graw *et al.*, 1979; Jakobsen and Nielsen, 1983, Saif and Khan, 1977). Yet this study shows that wheat yields can be considerably increased by VA mycorrhizal infection.

In this investigation the sources of P were two forms of fertilizer P, $FePO_4$ and monocalcium phosphate (MCP). The $FePO_4$ treatment represents an extreme P deficiency so that the wheat plants remained small and did not tiller. On one hand VAM is most effective and accelerates the plant growth, when P supply in soil is limited (Mosse, 1973), such as with $FePO_4$ fertilization (Bolan *et al.*, 1987). But on the other hand tillering is an important yield component in wheat and the mycorrhizal efficiency is estimated by its effect on the yield of the host plant. Therefore more screenings under moderate P deficiency are necessary.

In the screening and comparison of P and F_1 generations strong variations in infection rate and total infected root length among the wheat genotypes were observed. The mycorrhizal efficiency was affected by the P uptake. And this was positively correlated with the total infected root length. Azcòn and Ocampo (1981) found different mycorrhizal infection and dependence in wheats inoculated with *Glomus mossae*. They screened 13 wheat cultivars, but found no relation between mycorrhizal dependence and P concentration in plant tissue.Jakobsen and Nielsen (1983) found a negative correlation in cereal crops between infection rate and root density. Jensen (1982) found no relationship between infection rate and VA mycorrhizal efficiency in barley, but Iqbal *et al.* (1978) did so with infection intensity (number of arbuscles) in wheat.

Pinthus and Eshel (1962), McKey (1973) and Derera *et al.* (1969) studied the variation of the root volume in wheat and Monyo and Whittington (1970) analysed the genetics of some wheat root characteristics. There is a trend towards a mirror pattern between shoot and root, but the correlation is apparently loose enough that it allows considerable deviation (McKey, 1973). It is possible to breed for short or semidwarf wheat cultivars with an intense and effective root system.

Bertheau *et al.* (1980) screened spring wheat varieties for VA mycorrhizal efficiency and found an influence of dwarfing genes of Norin 10 wheat. Semidwarf wheat had the highest yield increase by VAM. Jensen (1985) quantified the variability among 398 pea varieties in root length, root dry weight and VA mycorrhizal development in relation to plant height and top dry weight. The results indicate, that high root length is linked only to a limited extent to unsuitable tall plants.

In agriculture 50 % of the yield increases in the last few decades is due to mineral fertilization (Von Peter, 1984). Today particular attention should be given to abiotic and biotic factors which affect the efficient utilization of fertilizers. The selection of new varieties is usually carried out under high nutrient conditions, and the high yields of the HYVs depend on a high mineral fertilization.

The VA mycorrhizal mycelium can form the majority of the soil biomass (Hayman, 1978),

primarily, in field soils with no or low mineral fertilizer and pesticide application. In soils with a high biological activity, the roots of cultivated plants are well infected by VAM (Steffan, 1985). Young (1985) made the assertation that on a high P level in the soil the VA mycorrhizal fungi are pathogenic inhabitants. This became true with MCP treatment in the screening experiment of 1985, when most of the wheat genotypes were parasized by *Glomus manihotis*, especially the HYVs. Wheat plants well supplied with P do not need VAM and the competition between fungus and host plant for carbohydrates can lead to parasitism (Bethlenfalvay *et al.*, 1982).

There exist strong genotype × environment interactions. For example with MCP fertilization *Glomus manihotis* acted parasitically in the year 1985 and enhanced the plant growth of both parental varieties, 'Huanca' and *Triticum aestivum rotundatum*, in summer 1986 with higher light intensity but the same experimental design.

Interactions between the genotype of the VA mycorrhizal fungus and the genotype of the host plant may be important for the plant growth response to VAM inoculation. In this study only one VA mycorrhizal fungus strain was investigated. Few plant families on the world are non-mycorrhizal and there seems to exist no vertical resistance within a genus against mycorrhizal penetration into root tissue. In the present study the infection rate varied strongly, indicating different susceptibility of a variety seems to be strongly influenced by environment and fungus used.

Therefore the 'Genetical Analysis of the Efficiency of VA mycorrhiza with Spring Wheat' requires further investigation before this symbiosis can be used in plant breeding for low-input varieties: first, the research of the inheritance modus in more reciprocal crossings between different genotypes, second, the test of some genotypes over several years to measure the genetical and environment-dependent variances and third, segregation analysis of the VA mycorrhizal efficiency in F_2- and backcross-generations to determine the number of genes involved. Different VA mycorrhizal fungi should be tested, to quantify the genotype fungus × genotype host plant-interaction. In hexaploid wheats monosomic lines exist, which can be used to detect gene loci on wheat chromosomes, responsible for the VAM action in wheat host plants (Röbbelen, 1968; Sears, 1958).

Acknowledgements

The author is deeply appreciated to Prof em, Dr S Rehm, Prof Dr W Achtnich and Dr A Moawad for their supporting this study. The financial support by the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

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PLSO PN 182
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Improved growth of *Cajanus cajan* (L.) Millsp. in an unsterile tropical soil by three mycorrhizal fungi

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Key words: *Cajanus cajan*, mycorrhizal fungi, P sources, Rhizobium

Abstract

Under glasshouse conditions *Cajanus cajan* plants grown in a dark red latosol were fertilized with soluble simple superphosphate and hardly soluble rock phosphate and inoculated with three VA mycorrhizal fungi (M_1, *Gigaspora margarita*; M_2, *Scutellospora verrucosa*; M_3, *Acaulospora rehmii*) from the Cerrado ecosystem, Brazil. Only with rock phosphate plant growth was significantly increased by all fungi. Enhanced P uptake corresponded with higher yields and proved to be a characteristic of the VA myccorhizae. A definite relationship between infection intensity and efficiency of VA mycorrhizae was not detected. Spore production was generally more pronounced in the treatment with rock phosphate, especially with M_1 and M_2. Nodulation of *Cajanus cajan* was greatly improved by all fungi in the treatment with rock phosphate. It is suggested that the increased plant development and nodulation was due to improved uptake of P by mycorrhiza.

Introduction

Restoration and maintenance of soil fertility is a basic and critical environmental problem. It is especially serious in tropical countries where nutrient-deficient soils are frequent. The use of expensive inputs such as mineral fertilizer to amend the deficiency is economically often not acceptable. In order to meet this limitation various agricultural practices are being tested at the Cerrado Research Center of Brasilia (CPAC). The introduction of legumes into crop rotation has recently been demonstrated to be a practicable way to increase crop production by improving soil fertility. Among the various leguminous candidates tested, *Cajanus cajan* L. has proven to be well adapted to the climate and to the phosphorus-deficient soil conditions of the Cerrado region of Brazil (Pereira, 1984). Schaaffhausen (1984) reported that due to its multiple use, *Cajanus cajan* could become an important legume for Central Brazil.

Nitrogen fixation by legumes is an effective economic way of providing N to subsequent crops especially for fragile farming systems in the tropics. However, nutritional factors may affect the legume-rhizobium symbiosis and Loneragan (1972) reported that phosphorus is emerging as the element most limiting growth of legumes in tropical environments. In this context investigations on the beneficial effect of VA mycorrhiza in improving P availability, plant growth and nodulation of some legumes were carried out by several researchers (Bagyaraj *et al.*, 1979; Crush, 1974; Ganry *et al.*, 1985; Guey *et al.*, 1987; Islam *et al.*, 1980; Mosse, 1977b; Saif, 1987; Yost and Fox, 1979). However, up to the present very little is known about the response of *Cajanus cajan* to VA mycorrhiza (Manjunath and Bagyaraj, 1984).

The aim of the present research work was to verify to what extent various indigenous VA mycorrhizae of the Cerrado ecosystem could improve plant growth of *Cajanus cajan* fertilized with two P sources of different solubility.

Materials and methods

The glasshouse experiment was conducted from beginning of June to beginning of September 1984 at the Cerrado Research Center of Brasilia (CPAC). The temperature ranged between 33°C during the day and 23°C at night. The soil used (not sterilized) had an indigenous endomycorrhizal spore population of 38 per 50 cm^3 soil (average of 10 replications) and the soil had the following properties: pH (KCl), 4.1; organic C (%), 2.1; exchangeable cations (meq 100 g^{-1} soil): Al, 1.75; Ca, 0.27; Mg, 0.08; K, 0.06; Al saturation %, 71; free Fe_2O_3 (%), 9.4; extractable P (Bray I), 1.2 mg kg^{-1}.

Following local agricultural practices the original pH was adjusted to 5.6 with dolomitic limestone and a basic fertilization was applied: 50 kg K ha^{-1} (KCl); 40 kg ha^{-1} of FTE BR 12, a fritted trace element mixture with the following contents (%): Zn, 1.8; Cu, 0.8; Fe, 0.3; Mn, 2.0; Mo, 0.1. For the P treatment two P sources at different levels were used: Rock phosphate (Patos de Minas) from Brazil (total P, 10.7%), 0, 200, 400 kg P ha^{-1}, and simple superphosphate (total P, 8%), 0, 50, 100 kg P ha^{-1}.

Cajanus cajan (L.) Millsp. was used as test plant and grown in plastic pots holding 2 kg soil with three seedlings per pot. The mycorrhizal inoculum consisted of a mixture of soil/roots/spores and was applied at the rate of 3 g pot^{-1}. All mycorrhizal fungi were propagated in the glasshouse using *Pueraria phaseoloides* (Roxb.) Benth. as host plant. The strains tested in this experiment were *Gigaspora margarita* (M_1), *Scutellospora verrucosa* (M_2), and *Acaulospora rehmii* (M_3) all from a virgin local soil. Two weeks after emergence of the seed all *Cajanus cajan* plants were also inoculated with a peat-based inoculum of native effective strains of Rhizobium, kindly provided by Dr Peres (CPAC).

Each treatment consisted of four replications. The pots were arranged in a randomized complete block design. Non-inoculated plants, designated as NI, served as control.

The plants were harvested after 110 days and the dry weights of the shoots were recorded. After clearing and staining the roots, the percentage mycorrhizal infection of the roots was determined by the gridline intersect method (Giovannetti and Mosse, 1980) and number of nodules were recorded. Number of VA mycorrhizal spores was counted in 50 cm^3 of soil using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Phosphorus concentration of the shoot was measured colorimetrically by the vanadomolybdate/phosphoric-yellow colour method described by Jackson (1973).

Treatment means were compared by using an analysis of variance (ANOVA), and where the F ratio was significant the least significant difference (LSD) was calculated for $P = 0.05$.

Results

Shoot dry weights of *Cajanus cajan* are presented in Figure 1. In general plant growth response to increasing P levels was found only at 200 kg P ha^{-1} in the rock phosphate treatment and at 50 kg P ha^{-1} in the simple superphosphate treatment. With exception of M_3 which was efficient only at 200 and 400 kg P ha^{-1}, all other mycorrhizal endophytes improved plant growth significantly at all P levels in the rock phosphate treatment. M_1 proved to be the most efficient fungus. In the superphosphate treatment plant growth of *Cajanus cajan* was not increased by any of the mycorrhizal fungi at high P levels. Data in table 1 and 2 indicate that P concentration was not modified by P source nor by any endophyte. However, P uptake of mycorrhizal plants fertilized with rock phosphate was significantly higher resulting in an improved plant growth.

The analysis of percent VAM infection and spore production are shown in Table 3. The highest mycorrhizal infection rates were recorded with M_1, M_2 and M_3 at 50 kg P ha^{-1} in the treatment with soluble superphosphate. With increasing levels of rock phosphate, however, infection rate tended to decrease slightly with NI and M_1, was not altered with M_2, but increased with M_3. Mycorrhizal spore counts indicated that the P source influenced spore number. With increasing P level of simple superphosphate spore production of NI, M_1 and M_2 was significantly reduced, most pronounced in M_1. With rock phosphate, spore number of M_1 was high at all P levels. With M_2, however, the significantly highest spore number was recorded at 400 kg P ha^{-1}. Number of nodules was in general higher with superphosphate than with rock

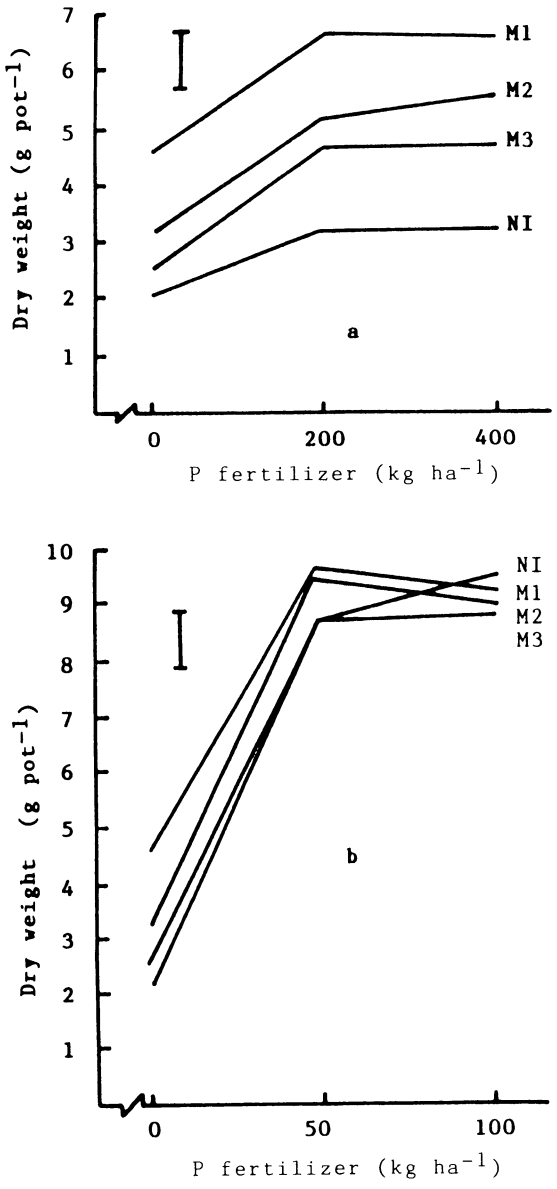

Fig. 1. Shoot dry weight (g pot^{-1}) of *Cajanus cajan* plants inoculated with three endomycorrhizal fungi and fertilized with different levels of **a**) rock phosphate and **b**) superphosphate. *Bars* indicate LSD at $P = 0.05$.

Table 1. P concentration (%) and P uptake (mg pot^{-1}) of *Cajanus cajan* inoculated with three endomycorrhizal fungi and fertilized with different levels of rock phosphate

Inoculation	P level	P conc. (%)	P uptake (mg pot^{-1})
NI	0	0.06	1.26
	200	0.06	1.94
	400	0.05	1.62
M_1	0	0.05	2.29
	200	0.08	5.36
	400	0.07	4.60
M_2	0	0.06	1.92
	200	0.07	3.64
	400	0.07	3.96
M_3	0	0.06	1.50
	200	0.08	3.76
	400	0.08	3.78
LSD ($P = 0.05$)		ND	1.73

ND, not determined.

Table 2. P concentration (%) and P uptake (mg pot^{-1}) of *Cajanus cajan* inoculated with three endomycorrhizal fungi and fertilized with different levels of superphosphate

Inoculation	P level	P conc. (%)	P uptake (mg pot^{-1})
NI	0	0.06	1.26
	50	0.06	5.24
	100	0.07	6.65
M_1	0	0.05	2.29
	50	0.07	6.72
	100	0.07	6.31
M_2	0	0.06	1.92
	50	0.06	5.64
	100	0.06	5.34
M_3	0	0.06	1.50
	50	0.07	6.10
	100	0.08	7.06
LSD ($P = 0.05$)		ND	2.21

ND, not determined.

phosphate (Table 4). In NI and M_1 formation of nodules increased with increasing P levels of superphosphate; however, with M_2 and M_3 differences between 50 kg ha^{-1} and 100 kg ha^{-1} were not detected. In the rock phosphate treatment number of nodules in NI was always very low at all P levels. With M_1 number of nodules increased only till 200 kg P ha^{-1}, whereas with M_2 and M_3 highest number of nodules was found at 400 kg P ha^{-1}.

Discussion

It is well known that legumes require relatively large amounts of phosphorus for optimum growth, nodulation and N_2-fixation (Asai, 1944; Bergersen, 1971; Carling *et al.*, 1978; Gibson, 1976; van

Table 3. Percentage mycorrhizal infection (+) and total number of spores (++) in 50 cm^3 of soil

Inoculation		Superphosphate (kg P ha^{-1})			Rock phosphate (kg P ha^{-1})		
		0	50	100	0	200	400
Not inoculated	+	43	46	34	43	30	20
	++	35	4	5	35	61	27
Gigaspora margarita							
	+	54	75	49	54	37	31
	++	103	40	11	103	120	113
Scutellospora verrucosa							
	+	45	61	36	45	37	50
	++	66	20	3	66	90	162
Acaulospora rehmii	+	22	54	37	22	29	44
	++	7	4	0	7	16	18
LSD (P = 0.05)		+ = 20	++ = 33		+ = 10 y	++ = 57	

Table 4. Number of nodules (number pot^{-1}) in *Cajanus cajan* as influenced by three endomycorrhizal fungi and different levels of phosphate from two P sources

Inoculation	Superphosphate (kg P ha^{-1})			Rock phosphate (kg P ha^{-1})		
	0	50	100	0	200	400
Not inoculated	4	50	73	4	7	10
Gigaspora margarita	20	46	73	20	53	41
Scutellospora verrucosa	7	68	56	7	29	40
Acaulospora rehmii	9	46	52	9	22	46
LSD (P = 0.05		22			23	

Schreven, 1958). Therefore, P fertilizers with different solubility differentially influence development of legumes especially under P-deficient soil conditions of the tropics. In this context VA mycorrhiza play an important role. The main contribution of VAM fungi to the host is to reach and translocate phosphate through their extracortical hyphae which would otherwise not be available to the host. However, the beneficial effect of VAM fungi to the tripartite symbiotic association can notably be modified by the P status in the soil. This was clearly demonstrated in the preceding results. In order to illustrate the key role of VA mycorrhiza on legume growth a schematic presentation is given in Figure 2.

In the treatment with rock phosphate all VAM fungi significantly enhanced shoot dry weight of *Cajanus cajan* when compared to NI. These results agreed well with improved P-uptake due to mycorrhiza inoculation. Comparable results with rock phosphate and cowpea were obtained by Islam *et al.* (1980) and Ames and Bethlenfalvay (1987).

In the presence of superphosphate shoot dry weights of NI and M plants were always equal and plant development corresponded with P uptake. Also using *Cajanus cajan*, Manjunath and Bagyaraj (1984) could observe improved plant growth with *Glomus fasciculatum* and superphosphate. Porter *et al.* (1978) found that applications of high levels of superphosphate did not affect growth of mycorrhizal clover plants. Considering that growth of *Cajanus cajan* with M_1, M_2 and M_3 was not negatively affected in the presence of superphosphate, it can be assumed that these endophytes were insensi-

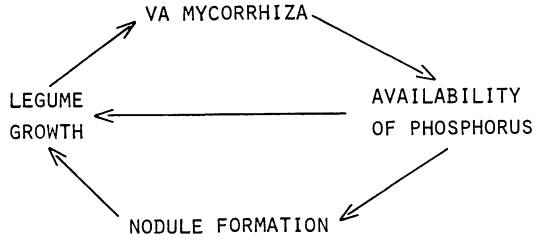

Fig. 2. Schematic presentation of the role of VA mycorrhiza in improving availability of phosphorus, nodule formation and growth of legumes.

tive to increasing levels of soluble P. VAM fungi adapted to low phosphate soils can differ in their reaction to added P fertilizers with different solubility (Clarke and Mosse, 1981; Mosse, 1977a; Powell and Daniel, 1978). Since *Cajanus cajan* was grown in an unsterile soil, inter-endophyte compatibility could explain why plant growth was not favoured by the fungi tested. This explanation is probably correct, especially when considering the results of Diederichs (1985) who found in the same but sterilized soil improved growth of *Cajanus cajan* with superphosphate only when inoculated with *Gigaspora margarita* (M_1) and *Acaulospora rehmii* (M_3).

In general, mycorrhizal infection was not much affected by any P fertilizer, although some differences between the VAM endophytes were observed. These results, however, can not be regarded as generally valid. Heckman and Angle (1987) found that large amounts of soluble P significantly reduced percentage colonization of VAM fungi and similar results were also reported by Sainz and Arines (1988). The internal P concentration of a plant, as a result of high P level in the soil, has been claimed as the factor controlling mycorrhizal colonization (Menge *et al.*, 1978). This, however, can not be valid for *Cajanus cajan* since P concentrations always were equal, either with superphosphate or with rock phosphate. Porter *et al.* (1978) under field conditions and Hicks and Loynachan (1987) obtained results indicating that increasing levels of superphosphate did not decrease the mycorrhizal population. Hayman (1982) reported that in soils which were very low in P small amounts of fertilizer did not reduce root infection, whereas at higher soil P levels, fertilization could decrease infection. These partly contradictory results show that the interaction P fertilizer/VAM infection may be rather complex especially under unsterile soil conditions.

Both P fertilizers influenced spore number of the three fungi differentially. Total number of spores were significantly decreased at higher rates of superphosphate. In contrast to these results Porter *et al.* (1978) reported that high rates of superphosphate did not reduce the population of large-spored VA endophytes of a fertilized pasture. With rock phosphate number of spores were not affected by higher levels of P. These findings lead to the assumption that mycorrhizal efficiency and spore production could be closely correlated in the presence of hardly soluble P at least when considering the results of M_1 and M_2.

With superphosphate, nodule formation was not influenced by any VAM inoculation treatment. Asimi *et al.* (1980) indicated that nodulation can be significantly enhanced by mycorrhiza at high P fertilizer levels. This, however, can only be valid for high-phosphorus tolerant VAM fungi which even in presence of large amounts of soluble P are able to promote plant growth (Davis *et al.*, 1984). The effect of P stress severely inhibited nodulation in the 0P treatment, and when plants were fertilized with rock phosphate and not inoculated with VAM. The positive influence of the tested fungi on nodule formation was well documented in the rock phosphate treatment, showing that all mycorrhizal fungi mobilized phosphorus from sparingly soluble rock phosphate not otherwise available for plant development or for nodule formation. Another important aspect of P stress is a decreased photosynthesis which may reduce mycorrhizal development and N_2 fixation. It is apparent from this study that VA mycorrhizae are indispensable for legumes when P is the main limiting factor for plant growth. The results of the present work clearly demonstrate an additive interaction of VA mycorrhiza and Rhizobium under P stress conditions resulting in: improved P uptake, increased nodule formation and, subsequently, enhanced plant growth. Considering the agricultural situation of the Cerrado region in Brazil one can say that *Cajanus cajan* has undoubtedly all the attributes to become an important legume especially as green manure for subsequent crops. In this context an important result should not be forgotten. A mycorrhizal study carried out at the CPAC Research Station (Reid *et al.*, 1987) revealed a significantly greater VA mycorrhizal infection in maize roots when either soybean or *Mucuna aterrima* were used previously as green manure. Similar results were also obtained by Sieverding and Leihner (1984). They observed that root infection of cassava with vesicular-arbuscular mycorrhiza was increased by crop rotation with grain legumes in the field in comparison to cassava grown in monoculture.

Acknowledgements

This research work was supported by the Inter-American Institute for Cooperation on Agriculture

(IICA) under a research contract with the Brazilian Agricultural Research Corporation (EMBRAPA). The author wishes to thank Professor Emeritus Dr S Rehm for his valuable comments and suggestions in reviewing the manuscript and Dr Peres for providing strains of Rhizobium.

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PLSO PN 183

Control of Mn status and infection rate by genotype of both host and pathogen in the wheat take-all interaction

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Key words: *Gaeumannomyces graminis*, genotypes, interaction, manganese, oxidation, take-all, *Triticum aestivum*, wheat

Abstract

Take-all is a world-wide root-rotting disease of cereals. The causal organism of take-all of wheat is the soil-borne fungus *Gaeumannomyces graminis* var *tritici* (*Ggt*). No resistance to take-all, worthy of inclusion in a plant breeding programme, has been discovered in wheat but the severity of take-all is increased in host plants whose tissues are deficient for manganese (Mn). Take-all of wheat will be decreased by all techniques which lift Mn concentrations in shoots and roots of Mn-deficient hosts to adequate levels. Wheat seedlings were grown in a Mn-deficient calcareous sand in small pots and inoculated with four field isolates of *Ggt*. Infection by three virulent isolates was increased under conditions which were Mn deficient for the wheat host but infection by a weakly virulent isolate, already low, was further decreased. Only the three virulent isolates caused visible oxidation of Mn *in vitro*. The sensitivity of *Ggt* isolates to manganous ions *in vitro* did not explain the extent of infection they caused on wheat hosts. In a similar experiment four Australian wheat genotypes were grown in the same Mn-deficient calcareous sand and inoculated with one virulent isolate of *Ggt*. Two genotypes were inefficient at taking up manganese and were very susceptible to take-all, one was very efficient at taking up manganese and was resistant to take-all, and the fourth genotype was intermediate for both characters. All genotypes were equally resistant under Mn-adequate conditions.

Introduction

Take-all, caused by the soil-borne fungus *Gaeumannomyces graminis* var *tritici* (*Ggt*), occurs wherever cereals and grasses are grown (Asher and Shipton, 1981). Scott and Hollins (1985) concluded that after decades of screening for resistance to take-all in cereals there was little hope for dramatic progress in resistance breeding. However, the severity of take-all is increased in host plants whose tissues are deficient for manganese (Mn) (Graham and Rovira, 1984; Huber, 1987; Willhelm *et al.*, 1988).

Significant interactions have been reported between the environment (in particular the nutritional status of the inoculum) and the virulence of *Ggt* isolates (Asher, 1981; White and MacIntyre, 1943). Very little work has been done on the importance of minerals in the nutrition and biology of *Ggt* (Sivasithamparam and Parker, 1981) and no work has been reported on differences in mineral requirements between *Ggt* isolates. The growth of many soil-borne micro-organisms (including fungi) have been found to increase with the addition of Mn to standard media (Casida and Thomas, 1961; Foster, 1939; Germida *et al.*, 1985) and many microorganisms can oxidize manganous ions (Mn-ions) to insoluble oxides (Bromfield, 1956; Bromfield and Skerman, 1950; Leeper and Swaby, 1940).

The effect of increasing concentration of Mn-ions in potato dextrose agar (PDA) on the growth and behaviour of four *Ggt* field isolates was tested in a petri-dish experiment. Two pot experiments

were conducted under controlled environment conditions to test the effect of the addition of Mn to a Mn-deficient soil on infection by the same four *Ggt* isolates.

Comparison of the independent screenings of a selection of Australian wheat cultivars and breeders' lines for tolerance to Mn deficiency (Graham, 1987; Graham pers. comm.; Marcar, 1986) and resistance to take-all (Penrose, 1985; Simon and Rovira, 1985; Wilson pers. comm.) shows that, in general, those cultivars which were tolerant of Mn deficiency (here termed Mn efficiency) were also more resistant to take-all (relative to other cultivars) and *vice versa*. The hypothesis that Mn efficiency confers resistance to take-all in wheat on Mn-deficient soil was therefore tested in a pot experiment which assessed the relative susceptibilities of 3 Australian wheat cultivars and 1 breeders' line to take-all under Mn-deficient and -sufficient conditions.

Materials and methods

Petri-dish experiment

Four different field isolates of *Ggt* (500, 47, 43 and 8), provided by Dr A Rovira, were tested for their sensitivity to increasing concentrations of Mn-ions *in vitro*. Mn-ion concentrations of 0, 25, 50, 100, 200, 400 and 800 mg kg^{-1} were prepared by adding appropriate amounts of a $MnSO_4$ stock solution to weak 'Difco' PDA (approximately 40% of manufacturers recommendations) which had been made up with double de-ionized H_2O. Disposable petri-dishes (9 cm diameter) were poured with 15 ml of agar (autoclaved at 120°C and 100 kPa for 15 minutes). Mycelial cubes (64 mm^3), removed from the colony edge of an actively growing culture on weak PDA (no added Mn), were used as inocula for test dishes. One mycelial cube was aseptically transferred to the centre of each dish and dishes were kept in the dark at 20°C for 5 days. Three dishes were prepared for each Mn concentration. Colony areas were calculated after 5 days from the average of 2 measurements of colony diameter taken at right angles to each other.

Pot experiments

Three pot experiments were conducted which separately tested the effect of soil-applied Mn on infection caused by four field isolates of *Ggt* and the effect of soil-applied Mn on *Ggt* infection of four wheat genotypes. Experiment A was factorially designed with 4 *Ggt* isolates (500, 47, 8, 43) and control, 2 Mn rates (0 and 4 mg of Mn per pot), and 4 replicates. Experiment B was a factorial combination of 3 *Ggt* isolates (500, 47, 8) and control, 3 Mn rates (0, 4 and 16 mg of Mn per pot) and 5 replicates. Experiment C was factorially designed with 4 wheat genotypes (Bayonet, Condor, Spear, C8MM), 3 Mn rates (0, 4, 16 mg of Mn per pot), 2 *Ggt* levels (−, +) and 5 replicates. The soil used in these experiments was a highly calcareous aeolian sand (Uc 1.11, Northcote 1979) from Wangary, Eyre Peninsula, South Australia. The soil in experiments B and C was incubated prior to sowing for two weeks at 18% water content (approximately 40% of field capacity) and 15/10°C day/night temperature to further decrease levels of available Mn in the soil (Leeper, 1970). Basal nutrients were mixed through the soil for each experiment at sowing at the rate of 2.37 mg K_2SO_4, 1.46 mg $MgSO_4.7H_2O$, 0.3 mg H_3BO_3, 0.2 mg $CuSO_4.5H_2O$, 0.5 mg $ZnSO_4.7H_2O$, 0.02 mg $CoSO_4.7H_2O$, 0.3 mg NaCl, 0.02 mg $H_2MoO_4.H_2O$, 9 mg $Ca(NO_3)_2.4H_2O$, 3 mg KH_2PO_4 and 0.4 mg $FeSO_4.7H_2O$ per plant. *Ggt* was added to the pots as a plug (same diameter as the pot) of 4% PDA + 1% 'Difco' Bacto-agar removed from the outer margins of an actively growing colony of *Ggt*, and placed at a set depth below the seed. Sterile agar plugs were used as controls for Ggt treatments. Condor seed was used in experiments A and B and all seed was from a Mn-deficient field site and surface-sterilized with sodium hypochlorite solution. Two plants were grown in pots 16 cm high, 2.5 cm top diameter with a 1° taper ('Pinecells', Ray Leach 'Cone-tainer' Nursery, Oregon, USA). The pots were partly filled with 42.5 g of soil at 25% water content (approximately 56% of field capacity), followed by an agar plug, a further 8.4 g of soil, 2 germinated seeds and a final layer of 4.1 g of soil. This arrangement placed agar plugs 2 cm below seeds. In Experiment C agar plugs were placed 4 cm below seeds (initial soil layers of 34.1 and 16.8 g, respectively). Pots from all experiments were kept in a controlled-environment growth room at 15/10°C (day/night temperature), a 10 hour photoperiod and a photon flux density of approximately 300 microEinsteins $m^{-2}s^{-1}$ for 25 days. Pots were watered to weight at regular inter-

vals. At harvest plants were washed free of the soil, scored for take-all (number of infected seminal roots, total number of black stelar lesions per plant and the total length of lesions per plant) and weighed. The shoots and roots were dried at 80°C for 2 days, weighed, digested in nitric acid and analyzed for manganese and 10 other essential elements with an ARL inductively coupled plasma atomic emission spectrometer. All data were analyzed with standard analysis of variance techniques appropriate to factorial experiments in randomized blocks.

Results

Petri-dish experiment

Increasing concentrations of Mn-ions in agar decreased the growth of isolate 500 and at 100 mg kg^{-1} of Mn-ions colony area was only half that without added Mn (Fig. 1). Oxidation of Mn-ions to insoluble brown oxides by 500 occurred at all rates of added Mn except 800 mg kg^{-1} where growth was completely inhibited.

Isolates 47 and 8 behaved similarly on PDA with increasing rates of added Mn but were not as sensitive to increasing Mn-ion concentrations as 500. The pattern and extent of Mn oxidation by 47 and 8 was very similar to that observed on 500 colonies.

Isolate 43 grew slowly on PDA (Fig. 1) and colony growth was stimulated by low concentrations of Mn-ions (up to 100 mg kg^{-1}). Concentrations of Mn-ions higher than 100 mg kg^{-1} decreased colony growth. Isolate 43 was a very poor oxidizer of Mn-ions on PDA.

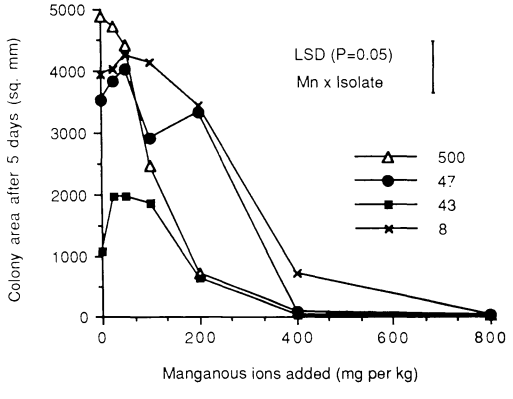

Fig. 1. Effect of increasing manganous ion concentration on growth of 4 *Ggt* isolates on potato dextrose agar at 20°C.

Pot experiment A

Isolate 500 caused heavy disease levels on wheat roots but 43 produced only low levels of take-all (Table 1).

Measurement of the number of black stelar lesions per plant showed that disease levels caused by *Ggt* isolates 500 and 43 were affected differently by Mn (Table 1). The number of lesions on plants infected by 500 decreased by 24% with Mn but on plants infected by 43 the small number of lesions was increased by 84%. Isolates 47 and 8 produced similar disease levels of 500 and were similarly affected by Mn but decreases with Mn were smaller and not significant in this experiment.

Similar trends were present in the total length of lesions per plant but were not statistically significant (Table 1).

Infection by isolates 500, 47 and 8 caused large depressions in host growth in both Mn treatments but decreases were most severe where Mn was added. Infection by 43 did not affect host growth (Table 1).

Plants grown without added Mn were Mn deficient and the addition of Mn increased the dry weight of shoots and roots (Table 1).

Shoot Mn concentrations in plants grown without added Mn were well below a critical level of 18 mg kg^{-1} established in experiments of a similar type (Wilhelm pers. comm.). The addition of Mn increased shoot Mn levels in all plants to well above deficiency but *Ggt* infection influenced the size of the increase (Table 1). The concentration of Mn in shoots of plants infected by 500, 47 and 8 were lower than in plants infected by 43, or disease-free. Infection by all four isolates increased root Mn concentrations in both Mn treatments (Table 1).

Pot experiment B

The total length of black stelar lesions per plant was decreased with the addition of 4 mg of Mn per pot, regardless of *Ggt* isolate type but there was no

Table 1. Experiment A. Effect of soil-applied Mn and *Ggt* isolates on *Ggt* infection, growth and shoot concentration of Mn in wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 8 plants

Mn added (mg pot)	*Ggt* isolate				
	500	47	8	43	Control
Total length of black stelar lesions (mm/plant)[a]					
0	31.0	49.9	43.9	10.9	0
4	26.9	32.2	38.5	17.8	0
LSD[b] ($P = 0.05$) Mn ns; Isolate = 9.9; Mn × Isolate ns					
Number of lesions per plant[a]					
0	9.5	9.2	8.3	2.4	0
4	7.3	7.7	7.9	4.4	0
LSD[b] ($P = 0.05$) Mn ns; Isolate = 1.3; Mn × Isolate = 1.8					
Percentage of infected seminal roots[a]					
0	100	93	93	50	0
4	97	98	95	56	0
LSD[b] ($P = 0.05$) Mn ns; Isolate = 7; Mn × Isolate ns					
Dry weight of whole shoots (mg plant^{-1})					
0	23	29	21	37	41
4	28	40	37	56	62
LSD[b] ($P = 0.05$) Mn = 4; Isolate = 7; Mn × Isolate = 9					
Dry weight of roots (mg plant^{-1})					
0	16	23	17	23	25
4	19	32	30	44	51
LSD[a] ($P = 0.05$) Mn = 4; Isolate = 6; Mn × Isolate = 8					
Mn concentration in whole shoots (mg kg^{-1} dry weight)					
nil	6.1	6.7	7.6	6.0	5.8
4	43.4	42.4	47.8	58.2	62.4
LSD[b] ($P = 0.05$) Mn = 2.6; Isolate = 4.1; Mn × Isolate = 5.8					
Mn concentration in roots (mg kg^{-1} dry weight)					
nil	18.3[c]	20.7	21.7	23.2	16.5
	(2.96)[d]	(3.08)	(3.12)	(3.19)	(2.86)
4	245	177	211	180	145
	5.51)	5.18	5.36	5.20)	4.98
LSD[b,e] ($P = 0.05$) Mn = 0.08; Isolate = 0.12; Mn × Isolate = 0.17					

[a] Disease measurements analyzed with control removed.
[b] Values separated by more than the appropriate LSD were statistically different.
[c] Geometric means calculated from natural log transformed data.
[d] Values in parentheses are natural log transformations, calculated to adjust for a skewed distribution.
[e] LSD's apply to transformed data only.

further decrease with 16 mg of Mn per pot (Table 2). The number of lesions per plant was not changed by Mn rates and effects of Mn on the percentage of diseased seminal roots were small and not consistent (Table 2).

When roots were washed free of soil at harvest the remnants of agar discs were still obvious. All inoculated agar discs from pots at 16 Mn were a dark brown colour. These discs stained dark blue with 0.1% benzidine in 1% ethanol which indicated that the brown colour was due to the presence of Mn (III, IV) oxides. Sterile discs from 16 Mn were

Table 2. Experiment B. Effect of soil-applied Mn and *Ggt* isolates on *Ggt* infection, growth and shoot concentration of Mn in wheat seedlings grown in a Mn-deficient sand under controlled environment conditions. Values in the body of the table are the average of 8 plants

Mn added (mg pot)	*Ggt* isolate			
	500	47	8	Control
Total length of black stelar lesions (mm/plant)[a]				
0	28.7	33.8	32.4	0
4	13.9	23.5	21.5	0
16	14.0	29.0	15.9	0
LSD[b] ($P = 0.05$) Mn, Isolate = 7.5; Mn × Isolate ns				
Number of lesions per plant[a]				
0	9.5	10.5	7.3	0
4	5.8	11.0	7.3	0
16	8.0	11.0	6.0	0
LSD[b] ($P = 0.05$) Mn ns; Isolate = 1.6; Mn × Isolate ns				
Percentage of infected seminal roots[a]				
0	98	96	88	0
4	74	98	98	0
16	93	98	82	0
LSD[b] ($P = 0.05$) Mn, Isolate ns; Mn × Isolate = 16				
Dry weight of whole shoots (mg plant^{-1})				
0	15.8	13.9	13.0	25.7
4	30.7	26.4	25.5	47.3
16	24.0	23.2	28.4	50.0
LSD[b] ($P = 0.05$) Mn = 22; Isolate = 2.5; Mn × Isolate = 5.2				
Dry weight of roots (mg plant^{-1})				
0	9.3	7.6	7.8	13.9
4	26.7	21.5	23.4	55.9
16	20.9	17.8	27.3	53.0
LSD[b] ($P = 0.05$) Mn = 2.6; Isolate = 3.0; Mn × Isolate = 6.1				
Mn concentration in whole shoots (mg kg^{-1} dry weight)				
0	5.8	6.5	5.6	5.6
4	24.8	24.3	30.1	36.6
16	84.1	76.9	105.2	115.9
LSD[b] ($P = 0.05$) Mn = 3.5; Isolate = 4.0; Mn × Isolate = 8.3				

[a] Disease measurements analyzed with control removed.
[b] Values separated by more than the appropriate LSD were statistically different.

not brown and only stained very lightly with benzidine.

Decreases in host growth due to infection by 500, 47 and 8 were similar to those measured in experiment A (Table 2).

The addition of 4 mg of Mn per pot caused a larger increase in host growth than in experiment A because the soil was incubated prior to sowing (Leeper, 1970) (Table 2). The high rate of Mn did not generally cause any further increases in host growth.

Shoot Mn concentrations at nil Mn were very deficient and well below the critical level of 18 mg kg^{-1} but all plants had adequate shoot Mn levels at 4 Mn (Table 2). Adding 16 mg of Mn per pot further increased shoot Mn concentrations to very high levels. Infection by all three isolates decreased shoot Mn concentrations in plants supplied with added Mn.

Experiment C

The effect of Mn on take-all depended on the wheat genotype involved (Fig. 2A).

Without added Mn, Bayonet supported an average of 25 mm of take-all lesions per plant, Condor and Spear 17 and 19 mm of lesions, respectively, and C8MM only 11 mm. The total length of lesions per plant was decreased by 4 Mn for all genotypes except C8MM which had a low level of disease at all Mn levels. Disease was approximately halved by 4 Mn for Condor and Spear and decreased by more than two-thirds for Bayonet. Disease levels were the same for all genotypes at 4 Mn and there was no additional decrease at 16 Mn.

The number of take-all lesions per plant and the percentage of infected seminal roots were not affected by treatments (data not presented). No disease was detected in − *Ggt* pots.

Ggt infection decreased the dry weight of shoots and roots of plants but the effects were most severe in pots where Mn had been added (Fig. 2B, 2C).

There were large visual differences between genotypes grown without added Mn. Bayonet was very pale green with strong interveinal chlorosis and some necrosis on young leaves. Condor also had strong interveinal chlorosis on young leaves but Spear was a darker green with only moderate interveinal chlorosis. C8MM was a healthy green and interveinal chlorosis on young leaves was only just evident. At 4 and 16 Mn all genotypes grew well and were a dark green except Bayonet at 4 Mn which showed early signs of interveinal chlorosis on young leaves.

The growth of plants at each Mn rate depended on the wheat genotype. Without added Mn C8MM had the highest shoot and root dry weights and responded least to the addition of Mn (Fig. 2B,

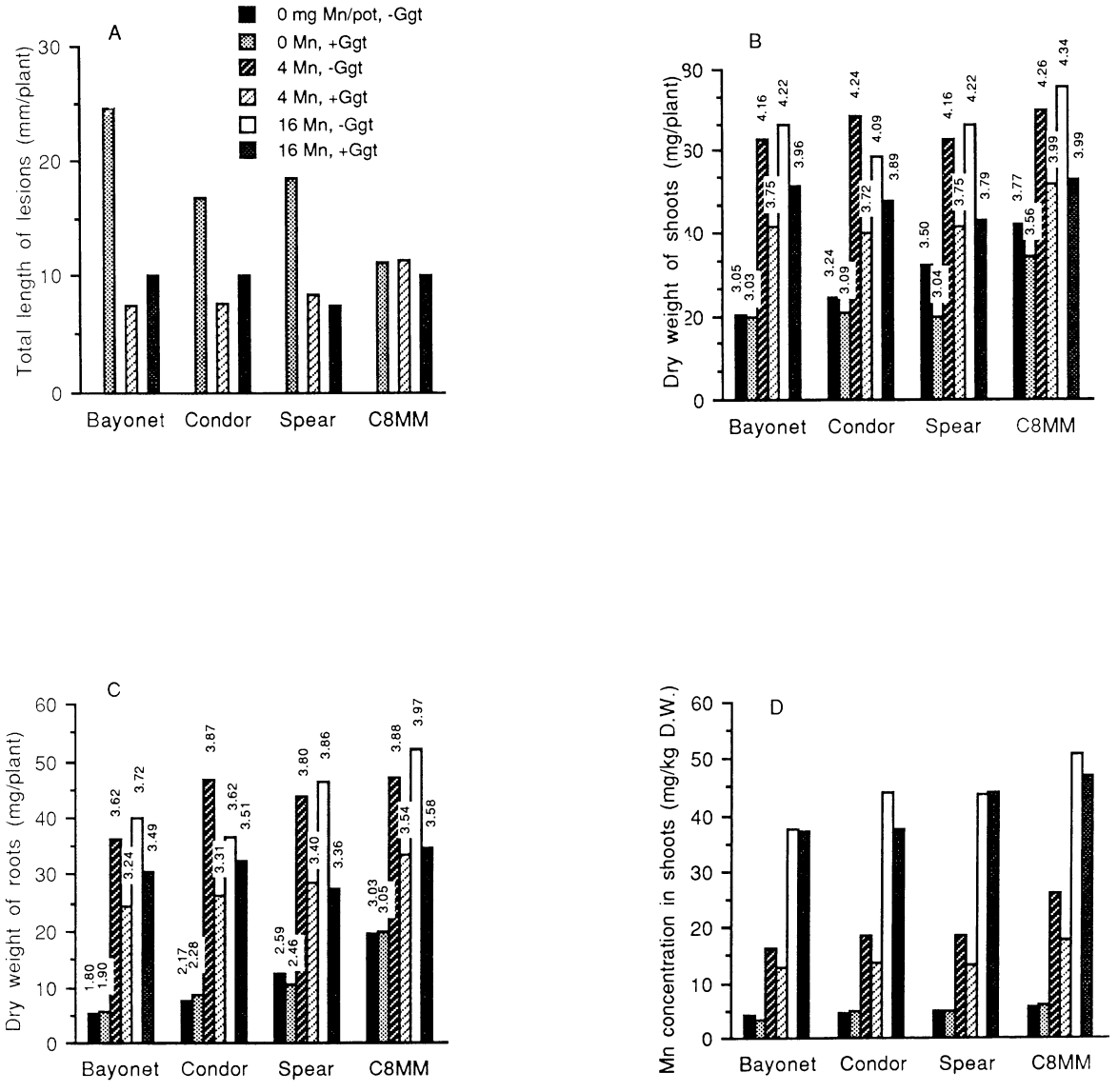

Fig. 2. Effect of soil-applied Mn and *Ggt* on total length of black stelar lesions (**A**) on seminal roots, dry weight of shoots (**B**) and roots (**C**) and shoot Mn concentration (**D**) in four wheat genotypes grown in a Mn-deficient sand under controlled environment conditions. Values are the average of 10 plants. Figures *above bars* in graphs B and C are natural log transformations to correct skewed distributions.

LSD's ($P = 0.05$) (apply to transformed data only for dry weight of shoots and roots). LSD's not presented were non-significant.

Graph A	Mn-	2.3	Graph B	Mn-	0.07
	Mn × Genotype-	4.5		Genotype-	0.08
				Ggt-	0.06
Graph C	Mn-	0.09	Graph D	Mn-	2.9
	Genotype-	0.10		Genotype-	3.3
	Ggt-	0.07		Ggt-	2.3
	Mn × Genotype-	0.17			
	Mn × Ggt-				

2C). Bayonet and Condor had the lowest shoot and root dry weights at 0 Mn and responded most to Mn. Spear was intermediate in size at 0 Mn and in response to Mn. The dry weight of shoots and roots of all genotypes were similar at 4 and 16 Mn.

The concentrations of Mn in shoots at harvest were well below adequate levels at 0 Mn for all genotypes and at both − and + *Ggt* (Fig. 2D) but both 4 and 16 Mn increased shoot concentrations to adequacy. *Ggt* infection had no effect on Mn concentration in shoots except at 4 Mn where it caused a small decrease in all genotypes. C8MM had higher shoot Mn concentrations than Spear and Condor in all treatments and they, in turn, had higher levels than Bayonet.

Discussion

Mixing low rates of Mn through soil decreased symptoms of take-all on roots infected by all 3 virulent Ggt isolates (virulent isolates produced black stelar lesions on infected host roots and depressed host growth).

In contrast to the depressive effect of soil Mn on infection by virulent isolates Mn stimulated infection by the weakly virulent isolate 43. This isolate infected wheat roots and produced characteristic black stelar lesions but at levels which were so low that host growth was not decreased. The growth of 43 on PDA also contrasted with that of the 3 strongly virulent isolates. Three features separated the behaviour of 43 on PDA from the other 3 isolates; very slow growth, a large stimulation in growth at low Mn concentrations and poor Mn-oxidizing ability. The first two features may help explain the increase in symptoms of infection by 43 with added Mn, given that infections by the 3 virulent isolates were decreased under the same conditions. The large stimulation in colony growth of 43 at low rates of Mn on PDA indicated that this isolate had a relatively high requirement for Mn. Infection of wheat roots at nil Mn by 43 may have been inhibited by slow hyphal growth due to insufficient levels of available Mn for normal hyphal growth. The rate of growth of runner hyphae down the outside of host roots is closely correlated with the length of infected roots (Garrett, 1936; Wildermuth and Rovira, 1977). Adding Mn to the soil eliminated deficiency in the host and perhaps also in 43 which may have increased the rate of hyphal growth of 43 (and consequently infection). This effect would not have been as important for the virulent isolates because their requirements for Mn on PDA were lower and they were fast growers at all Mn rates.

The observation that the 3 virulent isolates were strong oxidizers of Mn on PDA and in soil (Experiment B) but 43 was not suggests that Mn-oxidizing ability may be important in determining the virulence of individual isolates.

The sensitivity of isolates to toxic levels of Mn in PDA did not appear to be important to the extent of disease which infection by each isolate caused. Isolates 8, 43 and 47 had similar tolerances to increasingly toxic concentrations of Mn-ions but 500 was more sensitive. This pattern was not reflected in the virulence of each isolate in pot studies because infection by 47, 8 and 500 produced similar disease levels and all were decreased by soil-applied Mn to the same extent but infection by 43 behaved very differently.

The results of experiment C clearly showed that there was a strong and negative relationship between Mn efficiency of wheat genotypes and their relative susceptibility to take-all. C8MM produced more dry matter than Bayonet under conditions which were severely Mn deficient and accumulated a higher concentration of Mn in its shoots. Spear and Condor were intermediate between these two genotypes with Spear being more Mn-efficient than Condor. The ranking of these 4 genotypes for susceptibility to take-all under severely Mn-deficient conditions was in reverse order to Mn efficiency. The lowest total length of black stelar lesions per plant occurred on C8MM and the highest on Bayonet. The length of lesions on Spear and Condor was intermediate between C8MM and Bayonet and not significantly different from each other. All genotypes were equally resistant where Mn was applied to soil.

The largest decrease in total length of lesions with Mn also occurred on Mn-inefficient Bayonet and decreases on more efficient Spear and Condor were smaller. There was no decrease in length of lesions with added Mn in C8MM although C8MM was Mn deficient by harvest. The high Mn seed content of C8MM ($12.7\,mg\,kg^{-1}$ compared to 7.3, 9.2 and $8.6\,mg\,kg^{-1}$ for Bayonet, Spear and Condor, respectively) may have been the reason for

the absence of a decrease in total length of lesions with added Mn. Seed Mn is very important for early seedling growth (Marcar and Graham, 1986) and the seed reserves of Mn of C8MM may have been sufficient for growth during the time when invasion by *Ggt* was occurring and Mn-deficiency did not begin until after roots were colonized. All seed was taken from the same field site and the high content of Mn in C8MM seed may have been due to better growth of C8MM under Mn-deficient conditions or it may have been due to a superior ability of C8MM to translocate Mn to its seed.

Penrose (1985) reported that invasion by *Ggt* into seminal roots of Condor was slower than RAC311 (Bayonet) and that fewer stelar lesions developed on Condor. Similar differences in resistance between Condor and Bayonet were only evident under Mn-deficient conditions in this experiment. Further work is warranted to investigate whether the differences in resistance discovered here under Mn-deficient conditions can be explained by differences in rates of invasion and colonization by *Ggt*.

Graham and Rovira (1984) reported that a low rate of Mn which increased shoot Mn concentrations in wheat seedlings but did not eliminate Mn deficiency was partially successful in decreasing *Ggt* infection. The rate of 4 Mn in experiment C did not completely eliminate Mn deficiency in Spear and Bayonet (shoot Mn concentrations below critical) although no further increases in growth occurred with 16 Mn in either of these genotypes. This low rate of soil Mn decreased *Ggt* infection as effectively as 16 Mn on all four genotypes. The variable effects of low rates of Mn in decreasing *Ggt* infection highlights the subtle nature of the interaction between invasion of wheat roots by *Ggt* and rates of Mn which are marginal for growth of the plant host. Slight differences in Mn availability or timing and rates of Ggt invasion may be very important in determining the progress of the disease. These results suggest the possibility that Mn-efficient genotypes can substitute for fertilizer Mn in decreasing take-all in the field.

Acknowledgements

The first author was supported by a Commonwealth Postgraduate Research Scholarship and additional financial support was provided by the Commonwealth Wheat Research Council.

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Session 5

Germplasm resources and creation of genotypes for specific environmental including low input systems

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 425–430.
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PLSO PN 184

Genetic resources for optimal input technology — ICARDA's perspectives

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Key words: barley, dry areas, evaluation, fertilizers, genetic resources, indigenous germplasm, moisture stress, utilization, wheat

Abstract

With steadily increasing population pressure on natural resources, the primary challenge to agricultural research in the drylands will be to generate technologies which can sustain increased yields through judicious use of inputs and without risks of instability of production. The deployment of genetic resources to improve and stabilize crop production in the face of biotic and abiotic stresses is a key component in ICARDA's strategy to develop appropriate technology for stressed areas.

The high input technologies, while effective in areas for which they were developed, *i.e.* stress-free crop growing conditions, are rarely successful in the more severely stressed environments. Factors which reduce productivity such as drought, temperature extremes, salinity, low soil nutrients, diseases and pests are common in West Asia and North Africa. In the past breeders searched for desirable genes in exotic germplasm which may not be adapted to a given target environment. Relatively less effort has been devoted to producing cultivars that can provide stable yields under low to moderate input conditions. Landraces, primitive forms and wild progenitors could be used in reconstituting genepools of available genetic resources suitable for improving tolerance to stresses and minimizing losses through yield stability in low input dryland agricultural systems. The effects on cereal production of seeding rate, nitrogen fertilizer rate, use of different cultivars and timing of grazing by small ruminants have been studied and responses to various levels of chemical inputs have been measured. The exploitation of genetic variability for desirable traits within indigenous genetic resources which can fit in a specific agro-elecological zone is also reported here.

Introduction

The activities of the International Center for Agricultural Research in the Dry Areas (ICARDA) for winter cereals, food legumes and forages are oriented towards improving yield and production stability in drier, predominantly rainfed areas of West Asia and North Africa (WANA). The Center also has a wider global mandate for barley, faba beans and lentils in the developing countries. ICARDA is located in the proximity of one of the most ancient cross-roads of civilizations and surrounded by some of the oldest cultivated lands in the world (Bell, 1987). The WANA region encompasses the 'centers of origin and diversity' of important crop plants including barley, wheat, chickpea and lentils. In fact, their wild progenitors still grow as weeds in the vicinity of ICARDA's headquarters.

Crop improvement research during the last two decades has concentrated on environments which possess potential for high productivity such as irrigated areas and rainfed zones with favourable climatic conditions. The success of the green revolution in the 1960s and 70s boosted production of rice and wheat considerably in South and Southeast Asia and was underwritten by germplasms which were highly responsive to fertilizers, pesticides and herbicides. However, this high input technology has afforded little benefit to the moisture limited and low input environments. In fact serious questions are being raised about the energy-

intensive nature of these systems and their adverse effects on soil productivity and sustainability (Bidwell, 1986).

Several reports have discussed technology for the low input agricultural systems and provided suggestions for improvement of yield in stressed environments. At the time such technologies seemed very attractive since they promised increased yield with nil or almost negligible input requirements. On the other hand these technologies can at times lead to under-utilization of available natural resources which are limited. It is important to understand and determine appropriate levels of inputs which would be most economical as well as profitable to the farmer and at the same time not exhaust resources and degrade the environment in the long-term. It is essential to employ an optimal input strategy for sustained and increased productivity in a well defined environment rather than an excess or insufficient use of inputs. Although excessive utilization of inputs may lead to high yields in the short run, it can result in severe environmental degradation. Inappropriate or deficient inputs could also lead to non-optimal usage of resources and a consequent loss of production.

Despite the importance of durum wheat in the diet in WANA, where per capita consumption of wheat is the highest in the world at 150 kg year^{-1}, progress made so far to improve yields and stability of production particularly for durum wheat has been slow (Srivastava *et al.*, 1988). In the past, researchers have favoured bread wheat and its increased usage has pushed durum cultivation to more marginal areas and barley even further, often into areas which should not be under cultivation. For example, in 1909–10 season the wheat yield in Aleppo province of Syria was 1150 kg ha^{-1} when the area under cultivation for this crop was only 170,000 ha. By 1982–83 the yield had dropped to 965 kg ha^{-1} while the area of cultivation had increased to 214,000 ha (Gatchen, 1982). This paradox exists because cultivation under harsher environments has been on the increase in the province resulting in depressed yields. This has been the case all over WANA. Figures for area under cereal (wheat and barley) cultivation and yields over the years in four countries are given in Table 1.

Table 1. Area and grain yields of cereals (wheat and barley) in four countries during 1979–81 and 1985[a]

Country	Area cultivated (× 1000 ha)		Yield (kg ha^{-1})	
	1979–81	1985	1979–81	1985
Afghanistan	3254	3386	1340	1332
Iran	7877	8412	1137	1051
Pakistan	10693	11345	1609	1570
Sudan	4541	7229	666	564

[a] Source: FAO Production Yearbook Vol. 39, 1985.

The WANA environment

It has been estimated that 978 million hectares of land in WANA lies in the winter rainfall zone, and only 10–15% of this is thought to be arable. The soils of the region have a wide range which determines the quality of crop production. Combining this with estimates of the length of growing season only about 25% can be regarded as well suited for cultivation (vertisols, cambisols, and luvisols). A further 25% of xerosols could be utilized for crop production but must be considered as fragile and require special attention. Thus, it becomes clear that land with high yield potential (independent from water availability) is a limited resource in the region and ICARDA's perspectives are guided by these realities.

The climatic pattern in the region is predominantly mediterranean characterized by winter rainfall and hot dry summers. Precipitation is erratic and highly variable (100–600 mm) from year to year in the same area causing enormous fluctuations in crop production. The climates of the region form a continuum from sub-tropical mild conditions in parts of the lowlands of North Africa to the coldest plateaux of West Asia.

Predominant farming systems and agroecological zones in WANA

The three cereal crops, barley, durum and bread wheat are grown in distinct, although to some extent overlapping, environments which are defined in terms of growing days, integrating rainfall and temperature considerations (Table 2). Barley is the dominant crop in areas where rainfall is too low (< 300 mm) and unpredictable to raise a wheat or legume crop. Wheat is sown normally in

Table 2. The major agroecological zones of West Asia and North Africa (WANA)

Zones	Rainfall mm/annum	Crop growing days	Crop duration days
Very dry	< 300	60–140	100–200
Dry	300–400	110–170	130–210
Moderate rainfall	400–500	140–200	150–220
High rainfall	> 500	170–200	180–240
Highlands	250–500	70–180	200–360

NB: The growing day period concept considers temperature and water limitations.

areas receiving 300–600 mm rainfall. Durum wheat overlaps with barley at the lower end of this range, and bread wheat at the upper end.

Adjacent to the dry steppe, in areas where there is 200–350 mm of rainfall, livestock production continues to be the dominant occupation providing the bulk of farmers' income through dealings in dairy products, meat and wool. Here barley is by far the most important crop and is grown under low inputs with both grain and straw being utilized as feed for the livestock. Greater demand for livestock products in the region have forced farmers to cultivate increasingly on marginal lands which frequently results in overgrazing of the natural pastures. Continuous barley growing, eliminating the fallow, is detrimental to yield and threaten the natural resource base of the WANA region.

In the relatively higher rainfall areas (350–600 mm) wheat is the dominant crop. Bread wheat predominates in higher rainfall areas, but durum wheat forms the basic staple food in the drier areas of this zone. A wheat/fallow rotation is the norm, but in wetter areas and on better and deep soils rotation is practised with food legumes and summer crops such as melons, maize, sesame and cotton. Among the legumes chickpea predominates in the wetter areas and lentils in the drier parts. In the driest parts where rainfall is below 150 mm farming is only possible through irrigation, the source being surface or groundwater.

Optimal input technology

During the last decade there is increasing evidence to show that efficiency of winter cereal improvement for rainfed areas is greatly enhanced by exposing and selecting germplasm in environments where the product will ultimately be grown.

Low as well as high input technologies should be viewed in the light of target area and within the context of environmental limitations imposed on productivity potential. In order to increase and sustain production we should define specific environment as well as farming practices of the majority of growers.

Emphasis is being given to the development of germplasm and suitable agronomic practices for stressed environments as well as low input agriculture. To address these aims, the breeding programs have adopted approaches that allow identification of superior germplasm under stressed conditions. Special attention is given to the development of cultivars with stable resistance to insect pests and diseases.

It is considered desirable to employ integrated plant protection measures such as, use of genetically resistant cultivars, multiline cultivars and crop mixtures possessing resistance to a broad range of diseases and insects. It is also necessary to deploy crop rotations, farming practices, soil and crop management as well as careful use of herbicides, pesticides, insecticides and fungicides.

ICARDA emphasizes the need for better understanding of yield limiting stress factors in WANA such as drought, temperature extremes, salinity, low soil nutrients, diseases and pests imposed by major agro-ecological zones. This knowledge is particularly useful to 'tailor' genotypes to fit the targetted environment and farming practices. Selection techniques and methodologies have been developed and defined. For example, choosing different sowing times in order to expose the material under selection to various stresses at differing phenological stages and increased use of experimental designs better suited to handle environmental variations in the field. Also, under drought conditions where a crop relies increasingly on soil mositure, reducing some pre-anthesis water use and growth through selection of traits that reduce leaf area development could increase yields (Richards and Townley-Smith, 1987). Plant breeders are developing germplasm which can give reasonable yields in poor seasons but possess genetic ability to derive advantage from good seasons and produce considerably higher yields. Thus, a majopr objective is to prevent crop failures in years when stresses

such as drought, heat, cold, diseases and low rainfall prevail while maintaining the ability of the crop to respond to a better crop season.

There appears to be little doubt that crop genotypes can differ in various aspects of uptake and metabolism of mineral nutrients. In winter cereals for example, cultivar differences in grain yield response to applied nitrogen have been demonstrated in the field under a wide range of conditions (Krentos and Orphanos, 1979). Cultivar differences have also been observed in the use of nitrogen by the plant. Some genotypes of wheat and barley translocate most of the nitrogen for grain production whereas in others a considerable amount remains in the straw. Therefore it is imperative to assess the size and nature of diversity in genotypic response to nitrogen fertilizer in order that any practical differences could be exploited by the breeder (Anderson, 1985).

Efficiency of input use will have to be looked at more closely. This can be achieved through several means. Firstly, plant breeding can generate cultivars which make more efficient use of inputs such as phosphorus and nitrogen. Secondly, chemcial inputs can be complemented and partially replaced by biological processes which are less harmful to the environment. For instance, recent research has indicated that, in the long-term, organic farming systems are more effective in the drylands than conventional (intensive tillage farming) systems in reducing soil erosion and, therefore, maintaining soil productivity and sustainability (Reganold *et al.*, 1987).

Organic recycling and more tightly integrated crop/livestock systems can be used to minimize dependence on chemical inputs. Thus research on root fungi for nitrogen fixation could further reduce amount of fertilizers without sacrificing yields. Increases in the efficiency of crop nitrogen uptake or utilization in grain production, especially if accompanied by higher yield, could reduce input costs and risks for the farmers in rainfed areas. An example of risk avoidance strategy often used by farmers is to split nitrogen fertilizer applications. If crop prospects are poor in the spring costs can be minimized by reducing or dispensing with the second application.

Salinity of arable land is an increasing problem of many irrigated, arid and semi-arid areas of the region. Development of germplasm with increased tolerance to saline conditions and appropriate agronomic manipulations may be a solution for improved productivity. Hegla, a saline and moisture stressed site in northern Syria, is being used to screen diverse types of germplasm since 1982. Few accessions survived to maturity in each of the three replicates out of over a hundred planted. In addition lines 'Candealfen' from Argentina and 'Boohai' from Ethiopia performed well at Hegla for the third year (ICARDA, 1988).

Table 3. Grain yield (Q/ha) of improved cultivars and local landraces of barley grown on farmers' fields in 1986 season in Ethiopia under traditional and improved agronomic practices

Location	Variety or landrace	Traditional (low input)	Improved (high input)
Addis Alem	IAR/H/485	4.0	22.3
	A Hor 880/61	4.3	18.5
	Local	16.0	22.5
Wagetu	IAR/H/485	2.3	14.8
	A Hor 880/61	1.3	13.2
	Local	4.5	13.1
Besso	IAR/H/485	17.5	35.5
	A Hor 880/61	10.5	38.0
	Local	20.0	36.5

The low yield of barley in Ethiopia is mainly due to lack of adapted cultivars, abiotic and biotic stresses as well as poor agronomic practices. In a trial which compared traditional and improved practices of barley cultivation, local landraces performed better than cultivars developed for high inputs under traditional conditions. Even under improved optimal input conditions the landraces were able to perform as well as the improved germplasm (Table 3). However, increases in yield with the aid of fertilizers and improved agronomic practices could not be introduced to the farmers as they do not have access to fertilizers and other chemicals, nor are they in a position to adopt new methods.

Preliminary findings on evaluation of durum landraces and primitive forms of wheat indicate that it is possible to identify germplasm which may perform better, particularly under stressful rainfed conditions. Considerable genetic variability within populations of primitive forms of wheat for agronomic traits, *e.g.* growth habit, early vigour, drought tolerance, tillering capacity, crop duration, lodging susceptibility and grain quality, was identified during evaluation. For example, a number of

Table 4. Evaluation of primitive/wild forms of wheat at ICARDA for disease/pest resistance

Disease/pest	Primitive forms	No. of accessions
Yellow rust (*Puccinia striiformis*)	*T. dicoccum*	18
	T. carthlicum	2
	T. sphaerococcum	1
Common bunt (*Tilletia caries* & *foetida*)	*T. dicoccum*	13
	T. polonicum	2
	T. ispahanicum	1
	T. spelta	1
Oat bird cherry aphid (*Rhopalosiphum padi*)	*T. timopheevi*	1

accessions exhibiting resistance to some important diseases and pests of the region were observed at ICARDA (Table 4).

We are also increasingly turning towards wild relatives as these forms of genetic resources can withstand harsher environments having survived without domestication for centuries. For example, considerable variability was found in *Triticum dicoccoides*, the wild progenitor of durum wheat, for earliness, high protein content, tolerance to frost and yellow rust, and possibly for improved cooking quality (Damania *et al.*, 1988). Certain selected accessions of this wild species were crossed with *T. durum* at ICARDA in order to transfer desirable attributes to cultivated wheat (Tahir, 1983).

Hordeum spontaneum, the wild progenitor of cultivated barley, is being utilized in breeding programs aimed at developing genotypes with improved biomass and tolerance to biotic and physical stresses (Ceccarelli, 1984). Collections made in Cyprus and Syria indicate that local barley landraces may possess some traits of *H. spontaneum* as a result of intermediate forms gaining ground after *H. vulgare* × *H. spontaneum* gene exchange which occurs in nature (S Jana, pers comm, 1988).

Future research at ICARDA will focus on effective deployment of other wild members of Triticeae, such as *Agropyron* and *Aegilops* spp., in developing genotypes tolerant to prevailing stresses. In trials conducted with collaboration of Egyptian national program, two accessions of *Aegilops squarrosa* were found to be moderately resistant to oat bird cherry aphid (R H Miller, pers comm, 1988).

In conclusion it can be said that in breeding work at ICARDA special emphasis has been placed on adaptive traits which can cope with fluctuations in climate, water availability and soil fertility. There is a growing awareness that crop germplasm must possess specific characteristics which are aimed at target environments. It will be the Center's goal to develop optimal technology which does not overexploit natural resources leading at times to long-term degradation of the soil. On the other hand more effort is being made to produce germplasm which will possess ability to minimize the effect of abiotic stresses by utilizing indigenously available natural resources. The main objective is to develop genotypes which can fit a particular agro-ecological zone of the region. We propose to examine endemic genetic resources more carefully so that desirable genes relevant to low input agriculture can be found more readily rather than utilize exotic germplasm which is likely to be unadapted to the dry areas.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 431–448.
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PLSO PN 185

Physiological basis of differential response to salinity in rice cultivars

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Key words: genetic purification, growth analysis, leaf area ratio, rice seedling, root Na selectivity, salinity, salt tolerance screening

Abstract

Growth analyses of rice *Oryza sativa* L. seedlings in salinized nutrient solution condition were conducted with 24 cultivars and lines after genetic purification. Cultivar differences in relative growth rate in salinized conditions were chiefly dependent on differences in shoot Na content. The shoot Na content was affected by Na selectivity in the root and by the leaf area ratio (LAR, leaf area per total dry weight). The contribution of LAR was equally important to that of root cultivar selectivity against Na uptake under a higher salinization condition where root selectivity against Na may be decreased due to reduced root activity. Cultivar differences in salt tolerance in highly salinized conditions were mainly attributed to differences in these two factors. A more convenient and efficient screening method for salt tolerance is proposed.

Introduction

Rice *Oryza sativa* L. is considered moderately sensitive to salinity (Hayward and Bernstein, 1958; Maas and Hoffman, 1977). However, cultivars respond differently when subjected to salinity (Barakat *et al.*, 1971; Akbar *et al.*, 1972, De Datta, 1972).

Several factors contribute to salinity tolerance in rice. According to Yeo and Flowers (1983), entry restriction of sodium ions is a major contributing factor to salt tolerance, but initial salt uptake is responsible for only a certain amount of the variability. Other factors include differences in tissue tolerance to absorbed Na ions (Yeo and Flowers, 1983) and synchronization of ion compartmentation by the leaf cells with a high rate of ion transport to the shoot (Greenway and Munns, 1980).

Knowledge about the mechanism of salt tolerance in rice is still insufficient. Besides, significant differences in salt tolerance were observed with ordinary rice cultivars/line due to genetic impurity (Cabuslay and Akita, 1987), thus often causing difficulties in the analyses of results. Further study on the basic physiological mechanism that controls salt tolerance in rice will help breeders develop high-yielding, salt-tolerant cultivars. In this experiment, the growth characteristics of rice under salinized conditions were studied following genetic purification to understand the mechanism of salt tolerance and to propose a rapid and convenient procedure for screening for sality tolerance.

Materials and methods

Four R_3 generation lines derived from F_1 anther cultures produced by the Tissue Culture Facility in IRRI and twenty rice cultivars/lines consisting of well-known salt-tolerant cultivars like Pokkali and Nona Bokra, moderately tolerant ones like M242 and IR54 and susceptible cultivars like IR8 and IR50 were used in this study. Cultivars like Bg34, Bg90, Bg37 and IR5 were included in the study because of their vigorous seedling growth. Except Mahsuri and the anther culture lines, cultivars were used after genetic purification by the method proposed by Akita *et al.* (1987) to reduce variation in seedling tolerance to salinity within cultivars/lines.

In September 1987, six sets of 100 pregerminated seeds per cultivar/line were sown on a styrofoam

Table 1. Leaf area and dry weight of rice cultivars or lines grown at two salinity levels for 8 and 15 days

Cultivar or line	EC (mmho/cm)	8 days after treatment					15 days after treatment				
		Leaf area[a] (cm^2/plant)	Dry weight (mg/plant)				Leaf area (cm^2/plant)	Dry weight (mg/plant)			
			Leaf	Sheath	Root	Total		Leaf	Sheath	Root	Total
Pokkali (P)	Control	35.49	74	42	19	135	68.42	139	84	31	254
	8	28.60	62	34	18	114	44.68	113	72	31	216
	15	14.72	42	26	12	80	9.74	59	30	15	104
BR4-10 (BR)	Control	46.64	69	48	23	140	76.65	120	83	34	237
	8	31.18	56	47	29	132	43.46	91	85	41	217
	15	10.02	27	17	9	53	5.46	36	18	11	65
IR4595-4-1-13 (4595)	Control	36.12	68	44	20	132	55.59	98	64	26	188
	8	24.33	48	41	23	112	36.32	81	68	29	178
	15	11.41	34	20	11	65	4.31	43	21	12	76
KRI-24 (KR)	Control	37.10	52	29	17	98	64.61	99	63	30	192
	8	25.08	41	26	17	84	34.83	75	48	27	150
	15	6.04	25	13	8	46	0.20	25	11	7	43
Nona Bokra (NB)	Control	38.01	69	44	20	133	64.47	120	80	28	228
	8	28.77	58	42	23	123	54.33	110	100	38	248
	15	14.84	40	27	14	81	14.13	59	39	18	116
Bluebonnet 50 (BB)	Control	29.77	47	27	16	90	46.99	70	49	23	142
	8	17.38	31	22	13	66	16.07	47	35	17	99
	15	2.51	20	9	6	35	0.16	20	8	6	34
ADT 28 (ADT)	Control	47.38	76	47	19	142	73.84	136	88	28	252
	8	23.27	47	44	20	111	33.70	79	74	26	179
	15	10.94	38	23	10	71	1.45	42	19	10	71
Getu (G)	Control	38.28	66	40	11	117	68.73	120	78	37	235
	8	24.53	45	36	21	102	37.45	85	82	38	205
	15	11.90	34	23	13	70	6.08	48	27	14	89
M 242	Control	32.36	57	33	18	108	58.08	114	62	31	207
	8	20.62	45	30	19	94	34.48	75	51	24	150
	15	6.79	33	16	11	60	3.88	40	15	11	66
Annapurna (Ann)	Control	27.74	58	32	20	110	51.08	106	60	29	195
	8	17.10	36	22	16	74	17.36	63	39	22	124
	15	1.27	23	8	7	38	0.12	20	7	8	35
At 69-5 (At)	Control	35.84	62	33	16	111	70.00	117	68	24	209
	8	26.86	46	29	17	92	32.79	75	52	23	150
	15	10.47	37	20	12	69	4.34	47	20	13	80
Bg 34-8 (Bg34)	Control	32.61	52	35	17	104	55.40	91	61	23	175
	8	22.53	35	25	17	77	20.69	64	43	23	130
	15	2.88	22	10	8	40	0.38	26	11	8	45
Bg 90-2 (Bg90)	Control	29.75	45	27	15	87	64.65	106	66	29	201
	8	19.16	33	28	18	79	14.97	48	38	22	108
	15	3.63	24	12	9	45	0.32	23	10	7	40

Bg 367-4 (Bg367)	Control	29.51	52	31	29	112	61.24	99	57	25	181
	8	12.92	30	17	12	59	9.39	44	23	15	82
	15	0.35	16	7	5	28	0.01	15	7	5	27
IR5 (5)	Control	39.30	67	40	20	127	68.76	111	69	26	206
	8	25.64	41	36	21	98	17.80	55	54	25	134
	15	8.94	34	20	12	66	0.81	30	15	11	56
IR8 (8)	Control	35.16	64	38	20	122	67.54	110	69	28	207
	8	25.56	41	33	21	95	32.10	72	50	26	148
	15	8.95	33	17	11	61	0.41	29	16	9	54
IR36 (36)	Control	29.16	50	27	13	90	54.69	91	47	20	158
	8	16.32	34	23	14	71	22.15	60	34	19	113
	15	2.40	21	9	6	36	0.12	21	8	7	36
IR50 (50)	Control	33.57	55	30	15	100	67.17	96	53	20	169
	8	19.02	41	23	15	79	11.70	58	27	15	100
	15	0.44	18	7	5	30	0.02	15	6	5	26
IR54 (54)	Control	35.88	62	34	19	115	61.78	99	61	25	185
	8	25.17	43	26	17	86	29.01	71	42	23	136
	15	4.92	28	10	8	46	1.30	28	12	8	48
Mahsuri (Ma)	Control	24.84	40	26	14	80	43.06	74	51	21	146
	8	16.41	28	22	14	64	14.90	45	37	22	104
	15	5.51	18	10	7	35	1.08	20	10	7	37
AC6519-31 (AC19)	Control	35.48	61	37	18	116	70.71	129	80	32	241
	8	24.56	41	32	20	93	26.68	67	50	27	144
	15	8.14	28	16	11	55	4.26	39	18	13	70
AC6526-1 (AC26)	Control	34.04	62	36	21	119	70.66	120	73	34	227
	8	25.77	47	32	21	100	37.75	89	58	34	181
	15	10.84	32	20	12	64	3.60	38	16	12	66
AC6530-5 (AC30)	Control	30.94	56	32	18	106	62.50	117	70	35	222
	8	26.07	44	28	20	92	36.02	82	53	33	168
	15	11.36	35	20	14	69	6.84	49	21	15	85
AC6533-8 (AC33)	Control	35.86	82	47	25	154	72.88	182	111	41	334
	8	23.31	46	33	19	98	27.10	82	57	26	165
	15	9.63	36	20	13	69	2.72	41	20	12	73

[a] Living area.

Table 2. Percent Na in leaf, sheath and shoot of rice cultivar or line grown at two salinity levels for 8 and 15 days

Cultivar or line	EC (mmho/cm)	Na content (%)					
		8 days			15 days		
		Leaf	Sheath	Shoot	Leaf	Sheath	Shoot
Pokkali	8	0.24	1.19	0.57	0.37	1.56	0.83
	15	0.94	2.65	1.59	1.74	2.81	2.10
BR4-10	8	0.51	1.21	0.83	0.69	1.51	1.09
	15	1.16	2.48	1.66	2.20	3.61	2.67
IR4595-4-1-13	8	0.22	0.99	0.58	0.38	1.56	0.92
	15	0.79	2.27	1.33	1.60	2.61	1.94
KR1-24	8	0.39	1.16	0.69	0.65	1.67	1.05
	15	1.67	2.63	2.00	2.60	3.21	2.78
Nona Bokra	8	0.28	1.25	0.69	0.37	1.53	0.92
	15	0.80	2.35	1.42	1.26	2.31	1.67
Bluebonnet 50	8	0.61	1.57	1.02	1.57	2.66	2.04
	15	2.88	2.40	2.76	3.30	1.71	2.86
ADT28	8	0.31	1.02	0.66	0.61	1.74	1.16
	15	1.08	2.62	1.66	2.40	3.21	2.66
Getu	8	0.18	0.93	0.51	0.36	1.26	0.80
	15	0.56	1.81	1.07	1.40	2.71	1.87
M242	8	0.32	1.29	0.71	0.66	2.18	1.28
	15	1.26	2.62	1.71	1.94	2.51	2.11
Annapurna	8	0.73	2.09	1.24	1.27	2.89	1.89
	15	2.38	2.63	2.45	3.30	3.36	3.33
At69-5	8	0.71	2.24	1.31	0.90	2.42	1.53
	15	1.52	3.01	2.04	2.25	3.46	2.61
Bg34-8	8	0.33	1.16	0.68	0.58	1.67	1.02
	15	1.20	2.15	1.50	2.25	2.91	2.46
Bg90-2	8	0.54	1.49	0.98	0.84	2.09	1.38
	15	1.93	2.65	2.17	3.30	3.31	3.30
Bg367-4	8	0.97	2.44	1.49	1.46	2.74	1.90
	15	3.03	2.65	2.91	3.50	3.01	3.36
IR5	8	0.69	1.67	1.14	1.17	2.48	1.82
	15	2.22	2.97	2.48	3.15	3.21	3.16
IR8	8	0.61	1.71	1.09	0.84	2.48	1.51
	15	1.79	3.07	2.22	3.35	3.46	3.38
IR36	8	0.61	1.76	1.07	0.90	2.49	1.48
	15	2.27	3.17	2.57	2.90	2.71	2.86
IR50	8	0.91	2.42	1.45	1.39	3.49	2.06
	15	3.02	3.27	3.08	3.65	3.61	3.67
IR54	8	0.33	1.25	0.68	0.64	2.13	1.19
	15	1.40	2.67	1.74	2.07	3.46	2.50
Mahsuri	8	0.98	1.93	1.38	1.27	2.04	1.61
	15	1.86	2.31	2.00	3.55	2.61	3.23
AC6519-31	8	0.39	1.49	0.88	0.67	1.94	1.21
	15	1.34	2.68	1.84	2.15	3.41	2.54
AC6526-1	8	0.42	1.57	0.89	0.65	2.02	1.19
	15	1.41	2.61	1.87	2.45	3.04	2.63
AC6530-5	8	0.44	1.54	0.86	0.69	1.82	1.13
	15	1.17	2.55	1.67	2.25	3.36	2.59
AC6533-8	8	0.27	1.25	0.67	0.66	2.07	1.24
	15	0.75	2.20	1.27	1.71	3.11	2.16

seedbed floated on normal culture solution as recommended by Yoshida *et al.* (1976), with iron citrate replaced by iron EDTA at 2.4 ppm. The pH of the solution was adjusted daily to 5.0. The materials were grown inside the Phytotron glasshouse maintained at 29/21°C (day/night temperatures) and 70% relative humidity. After one week, two sets of uniform seedlings were transferred to a culture solution salinized at an electric conductivity (EC) of 8 mmho/cm; another two sets were salinized at 15 mmho/cm. After eight days of treatment, one set each of control and plants salinized with 8 and 15 mmho/cm were sampled. The remaining plants were sampled after 15 days of treatment. In each sampling, leaf area of green leaves was measured by a leaf area meter. The dry weight of each organ (root, leaf sheath and leaf blade) was then determined after oven-drying for 3 days at 70°C. The dried samples were finely ground for Na analysis. Sodium concentration was determined by an ion meter after extraction with deionized distilled water. Chemical analysis was done only for sodium ion because it was demonstrated by Mix (1973) that the harmful effect of NaCl was due to specific influence of Na^+ and not of Cl^-, since in a Na_2SO_4 medium identical damages of the chloroplasts were observed. On the other hand, chloride is best considered an inert anion which is tolerated over a wide concentration range (Clarkson and Hanson, 1980). Transpiration rates of Pokkali and IR36 in the control and in salinized conditions wre determined simultaneously by the decrease of culture solution during 4 days of treatment.

To compare the effects of salinization on plant growth, relative growth rate (RGR: growth efficiency per dry weight basis), net assimilation rate (NAR: growth efficiency per leaf area basis) and leaf area ratio (LAR: leaf area per total dry weight) for each period of treatment under different levels of salinization were calculated.

Results and discussion

Effect of salinization on growth characteristics

The decrease in dry weight and leaf area relative to the control were greater when salt concentration was increased from 8 mmho/cm to 15 mmho/cm and when seedlings were treated longer under each salination condition (Table 1). Sodium concentrations in the leaf and sheath were greater at higher salinization level and longer treatment duration (Table 2).

In general, the RGR decreased sharply as shoot Na concentration increased (Fig. 1, Table 3). This pattern of decrease differed with cultivar/line. At EC = 8 mmho/cm, RGR of cultivars like Annapurna, Mahsuri, and Bluebonnet 50 (with lower RGR in the control) increased or remained at similar levels as those in the control. However, cultivars with higher RGR in the control showed a continuous decrease in RGR with increased shoot Na content. This suggests that cultivars with higher growth rate in the control will be affected severely

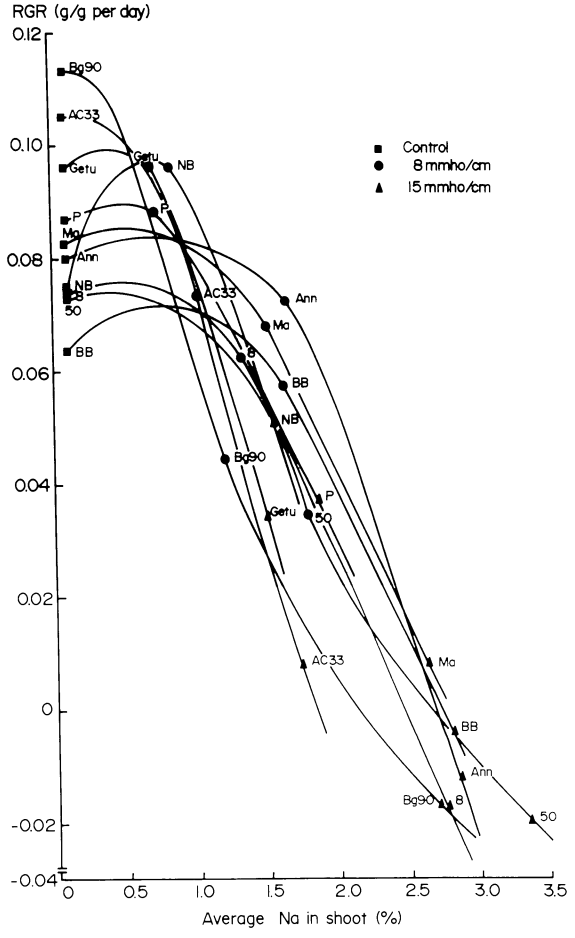

Fig. 1. Effect of Na concentration in the shoot on relative growth rate (RGR) of representative cultivars. RGR was calculated starting from 8 to 15 days after salinization. Average shoot Na concentration was obtained in the corresponding period.

Table 3. Values of RGR, NAR, LAR, and % survival at different EC levels from 8 to 15 days after salinization

Level of[a] tolerance	Cultivar or line[b]	EC (mmho/cm)	RGR (g/g/day × 10^{-3})	NAR (g/dm^2/day × 10^{-3})	LAR (dm^2/gTDW)	% Survival
High						
	Nona Bokra	Control	75	27	2.83	
		8	96	43	2.23	100
		15	51	35	1.48	99
	Pokkali	Control	87	33	2.66	
		8	88	40	2.21	100
		15	37	28	1.32	98
	AC6519-31	Control	100	34	2.98	
		8	61	28	2.15	100
		15	34	35	0.98	–
	Getu	Control	96	32	3.05	
		8	96	48	2.02	100
		15	34	30	1.13	95
	AC6530-5	Control	101	36	2.85	
		8	84	35	2.40	100
		15	30	25	1.20	–
	BR4-10	Control	74	23	3.29	
		8	70	33	2.15	100
		15	29	22	1.31	92
	IR4595-4-1-13	Control	50	17	2.87	
		8	65	31	2.09	100
		15	22	20	1.10	98
	At69-5	Control	87	27	3.28	
		8	68	28	2.45	100
		15	21	21	0.99	97
Intermediate						
	Bg34-8	Control	73	23	3.17	
		8	73	35	2.09	100
		15	17	44	0.39	19
	M242	Control	190	31	2.88	
		8	66	29	2.28	100
		15	14	16	0.87	81
	AC6533-8	Control	105	47	2.22	
		8	73	38	1.92	100
		15	8	9	0.86	–
	Mahsuri	Control	83	28	2.99	
		8	68	37	1.86	100
		15	80	9	0.92	41
	IR54	Control	67	21	3.27	
		8	64	26	2.42	100
		15	6	9	1.65	36
	AC6526-1	Control	89	30	3.02	
		8	82	36	2.25	100
		15	4	4	1.00	–
	ADT 28	Control	80	26	3.09	
		8	67	34	1.96	100
		15	0	0	0	85
	IR36	Control	78	23	3.36	
		8	65	31	2.08	100
		15	0	0	0	20
Low						
	Bluebonnet 50	Control	64	19	3.30	
		8	57	28	2.02	100
		15	−4	−11	0.37	92

Table 3. continued

Level of[a] tolerance	Cultivar or line[b]	EC (mmho/cm)	RGR (g/g/day × 10^{-3})	NAR (g/dm^2/day × 10^{-3})	LAR (dm^2/gTDW)	% Survival
	Bg367-4	Control	67	22	3.09	
		8	47	30	1.59	92
		15	−5	−79	0.06	7
	KRI-24	Control	93	26	3.52	
		8	81	32	2.57	100
		15	−10	−14	0.70	27
	Annapurna	Control	80	31	2.60	
		8	72	42	1.73	100
		15	−12	−62	0.19	11
	Bg90-2	Control	113	35	3.28	
		8	44	24	1.81	100
		15	−17	−36	0.47	43
	IR8	Control	74	24	3.14	
		8	62	26	2.36	100
		15	−17	−21	0.79	30
	IR50	Control	73	20	3.72	
		8	34	20	1.74	100
		15	−20	−248	0.08	0
	IR5	Control	68	21	3.25	
		8	44	27	1.65	100
		15	23	−29	0.78	32

[a] Tolerance was evaluated by RGR in 15 mmho/cm since tolerance at this concentration is more important for practical screening.
[b] Italicized cultivars showed consistently high or low salt tolerance at 8 and 15 mmho/cm.
[c] Due to impurity of anther-culture lines, survival values were not taken.

by salinity; their sensitivity to increased Na concentration in the tissue will be higher. However, when shoot Na content was high (at EC = 15 mmho/cm), differences in RGR among cultivars/lines at a certain shoot Na level was not in the same order as that when shoot Na content was low (Fig. 1). Cultivars with high salt tolerance like Pokkali, Nona Bokra and Getu had less shoot Na concentration at 15 mmho/cm (Tables 2 and 3, Fig. 1). Shoot Na concentration in high salinity condition therefore is an important factor that determines differences in salinity tolerance among cultivars.

To determine how RGR is affected by shoot Na content, values of NAR and LAR were plotted against shoot Na concentration. (RGR is defined as the product of NAR and LAR.) When shoot Na concentration was less than 1.5%, NAR in the treated condition was slightly higher than that in the control. However, beyond 1.5%, NAR decreased sharply in all cultivars/lines (Fig. 2). Cultivars with higher NAR in the control showed a faster rate of decrease, *i.e.*, the materials showing active growth with higher photosynthesis suffered greater damage from salt stress.

On the other hand, LAR showed a continuous decrease with increasing shoot Na concentration (Fig. 3). A lower RGR in salinization condition therefore can be attributed to both reduced NAR and reduced LAR which were mainly due to decreased leaf area development (Figs. 4 and 5).

Factors affecting Na uptake

Significant differences were observed in the amount of Na taken up by the plants among cultivars/lines. However, the Na content in each organ at 15 mmho/cm compared to that at 8 mmho/cm, and the Na content after 15 days of treatment compared to that at 8 days, showed an almost proportional relationship regardless of cultivar/line (Fig. 6). The rate of Na uptake increased almost proportionally with salinization concentration and treatment duration except in extremely high and long treatments where plants started to die. These results indicate that Na uptake of the rice plant is a rather physical process dependent on the Na concentration gradient, duration of treatment and

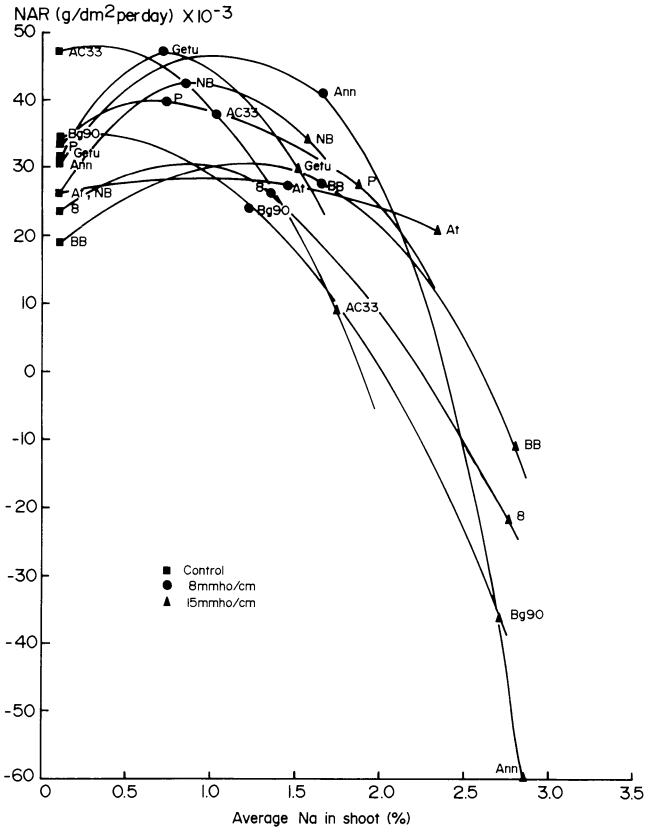

Fig. 2. Effect of Na concentration in the shoot on net assimilation rate (NAR) of representative cultivars. NAR was calculated starting from 8 to 15 days after salinization.

shoot dry weight. Therefore, the total Na uptake rate or Na/day at a certain concentration gradient of Na (Na) was obtained to determine the mechanism of Na uptake.

When the relation between (Na) and average leaf area under various treatments were obtained, (Na) generally showed a high positive correlation with average leaf area (Fig. 7). The values of (Na) of salt-tolerant cultivars like Pokkali, Nona Bokra, and Getu under different treatments were almost linearly related with evapo-transpirational surface *i.e.*, (Na) per unit leaf area (α) was almost constant in 8 mmho/cm (Fig. 7), although there were significant differences in α values among cultivars/lines. Most tolerant cultivars like Pokali, Nona Bokra, and Getu showed lower α values while susceptible cultivars like IR50, IR5 and Bg90 showed higher α values which increased with increased salinization level and prolonged treatment (Fig. 7 and Table 4). The higher transpiration rate of susceptible IR36 in 8 mmho/cm than that of Pokkali (Table 5) would partly be the cause for higher α value of IR36 than Pokkali in 8 mmho/cm. The high correlation between the amount of Na uptake and leaf area observed in this experiment and the reported higher effect of air humidity on salt uptake (Ota and Yasue 1959) indicates that Na uptake is partly dependent on passive absorption, affected by evapotranspirational area.

The change in α value among different treatments indicates the participation of other factors aside from evapotranspiration or evapotransiration efficiency per unit leaf area. The average transpiration rates of Pokkali and IR36 in the control were 18.25 and 15.37 g/dm^2/day, respectively (Table 5). However, in 8 mmho/cm transpiration rate of Pokkali decreased to 58% of the control while that of IR36 decreased only to 78% of the control. In 15 mmho/cm, transpiration rates decreased to 40–50% in both cultivars. The

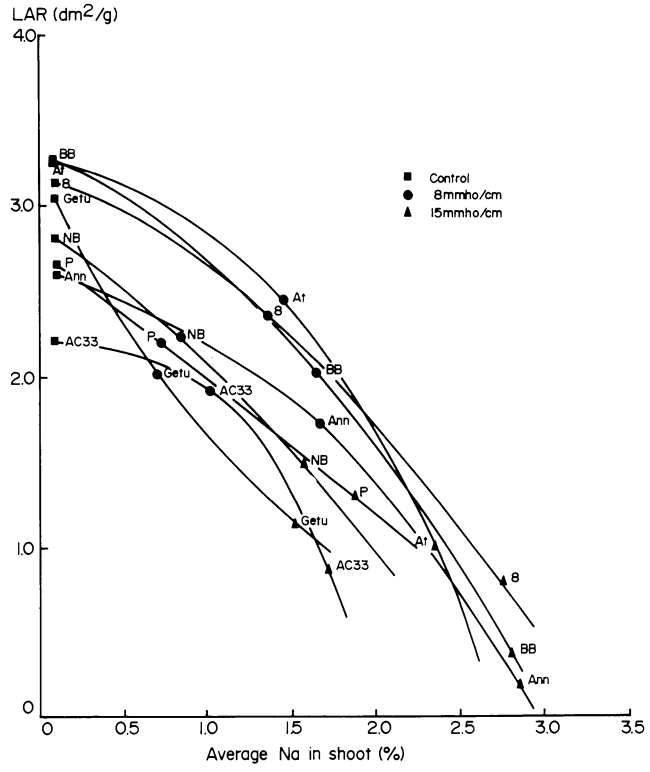

Fig. 3. Relationship between leaf area ratio (LAR) and average Na concentration in the shoot of representative cultivars. LAR was calculated using the formula RGR = NAR × LAR.

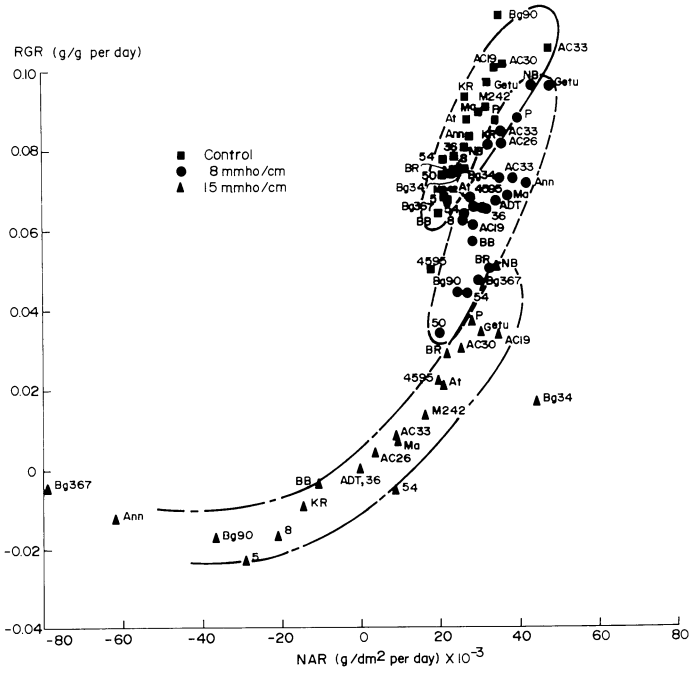

Fig. 4. Effect of NAR on RGR at two different salt concentrations.

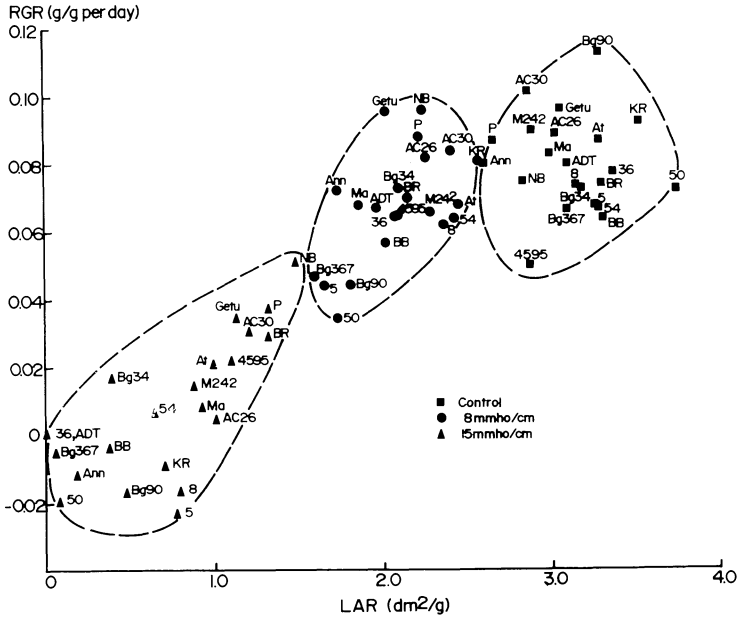

Fig. 5. Effect of LAR on RGR at two different salt concentrations.

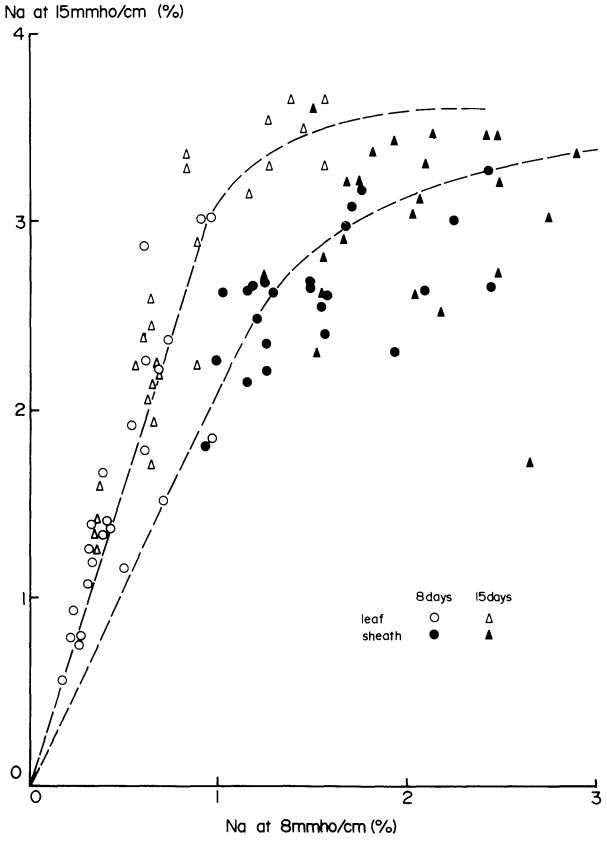

Fig. 6. Relationship between Na absorbed in leaf and sheath at 15 mmho/cm culture solution and Na content in leaf and sheath at 8 mmho/cm culture solution during 8 days and 15 days treatment. Each point in the figure was obtained with different cultivars.

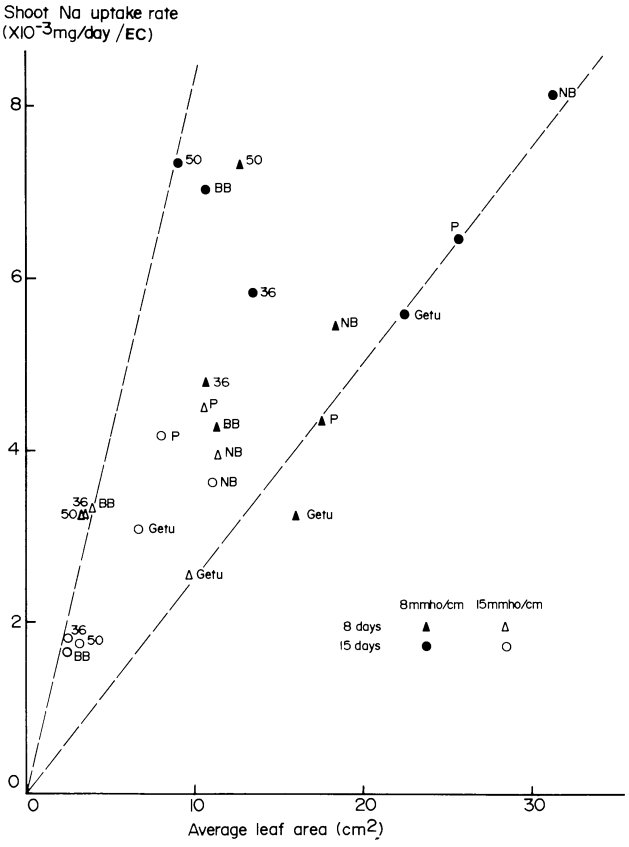

Fig. 7. Na uptake as a function of average leaf area at two NaCl concentrations in solution and at two durations of treatment in representative cultivars. Leaf area was calculated as the average between leaf area before treatment and leaf area after each duration of treatment.

osmotic adjusting process was rapid and stomata were partially open even in salinized condition although incipient wilting was observed in 15 mmho/cm. When Na uptake rate from each salinized solution is obtained (assuming Na ion is taken up only by evapotranspirational flow without interference by root selectivity) Na uptake rate of IR36, which showed higher transpiration rate than Pokkali in salinized condition at 8 and 15 mmho/cm can be calculated as 18.83 and 26.83 mg/dm^2/day, respectively. The actual amount of Na absorbed by IR36 in 8 and 15 mmho/cm for the first 8 days from treatment was 0.71 and 2.60 mg/dm^2/day, respectively. Therefore the actual Na uptake rate of IR36 in 8 and 15 mmho/cm was only 1/26 and 1/10, respectively of the calculated uptake rate which was estimated from transpiration rate. Furtherfore, sodium uptake at 15 mmho/cm of both cultivars showed significant differences (Table 4) although transpiration rate was almost the same and the actual uptake rate in 8 mmho/cm in which transpiration rate was higher was lower than those in 15 mmho/cm.

The lower actual uptake rate of Na may be mainly due to the high selectivity against Na ion at the root. The observations about increased salt tolerance of tomato and reduced shoot Na content after grafting with tolerant eggplant root (Tsuchiya *et al.*, 1987a) and decrease in Na and Cl concentrations in the shoot with a decrease in Na/Ca ratio (increase in Ca concentration) in the saline solution reported by Muhammed *et al.*, (1987) support the importance of root selectivity against sodium ion. The increased α value under prolonged treatment and high salinity level in susceptible cultivars/lines observed in this experiment may chiefly be attributed to increased root permeability to Na. This increase in root permeability might have been high enough to mask the effect of stomatal closure which should have decreased the α value.

Table 4. α values at different EC levels from 0–8 days after salinization

Level of[a] tolerance	Cultivar[b]	EC (mmho/cm)	α (mg Na/dm²/day/EC × 10^{-3})	Level of tolerance	Cultivar	EC (mmho/cm)	α (mg Na/dm²/day/EC × 10^{-3})
High				Intermediate			
	Nona Bokra	8	29		IR54	8	22
		15	35			15	43
	Pokkali	8	24		AC6526-1	8	34
		15	42			15	48
	AC6519-31	8	32		ADT28	8	28
		15	46			15	39
	Getu	8	20		IR36	8	45
		15	26			15	87
	AC6530-5	8	30	Low			
		15	43		*Bluebonnet 5*	8	37
	BR4-10	8	33			15	83
		15	32		*Bg367-4*	8	61
	IR4595-4-1-13	8	25			15	104
		15	31		KRI-24	8	22
	At69-5	8	46			15	49
		15	57		Annapurna	8	49
Intermediate						15	87
	Bg34-8	8	22		*Bg90-2*	8	40
		15	43			15	80
	M242	8	31		IR8	8	40
		15	55			15	62
	Ac6533-8	8	28		*IR50*	8	57
		15	37			15	92
	Mahsuri	8	50		*IR5*	8	40
		15	44			15	62

[a] Tolerance was evaluated by RGR in 15 mmho/cm since tolerance at this concentration is more important for practical screening.
[b] Underlined varieties showed consistently high or low salt tolerance.

Decreased root activity in salinized condition observed by Akbar *et al.* (1987) might have caused reduction in root Na selectivity and increased Na uptake into the plants of susceptible cultivars/lines.

Sodium uptake into the rice plant is therefore dependent on passive and active processes which are determined by leaf area and root selectivity, respectively. The Na uptake process can be summarized by the following equation:

$$\text{Na uptake rate/day (Na)} = \alpha \cdot \text{EC} \cdot \text{day} \cdot \text{LA} \quad (1)$$

$$\text{Let } \beta = \alpha \cdot \text{EC} \cdot \text{day}$$

$$\text{Total amount of Na uptake} = \beta \cdot \text{LA} \quad (2)$$

where (Na) is the total amount of Na uptake in shoot per day and LA is the total leaf area per plant. When both sides of equation (2) are divided by total dry weight, the equation can be shown as:

$$\text{Na content} = \beta \cdot \text{LAR} \quad (3)$$

where β is given by Na (g) per dm^2.

Participation of LAR on shoot Na concentration can be expected because larger evapotranspirational surface of plant relative to its tissue volume may cause a higher increase in Na concentration in the tissue even when the amount of Na uptake from the root is the same. Reduced root selectivity against the sodium ion in highly salinized condition will naturally cause higher dependency of sodium

Table 5. Transpiration rates of Pokkali (salt-tolerant) and IR36 (salt-susceptible) in the control and in salinized conditions[a]

Cultivar or line	Transpiration rate (g $H_2O/dm^2/day$[b])				
	Control	8 mmho/cm		15 mmho/cm	
Pokkali	18.25[a]*	10.51[b]	(0.58)	7.09[c]	(0.39)
IR36	15.37[d]	11.95[e]	(0.78)	9.08[f]	(0.59)

[a] Values in parentheses are relative to that in the control.
[b] Measured by the average decrease of culture solution within four days in glasshouse whose day and night temperatures were 29°C and 21°C, respectively; average of three replications.
[c] All means are significantly different at 5% level according to Duncan's multiple range test.

Table 6. Multiple linear regression analysis between shoot Na content and two independent variables α and LAR at 8 days treatment with different Na concentrations in culture solution

Na concentration of culture solution (mmho/cm)	R	R^2	Rate of SPRC (%)	
			X_1	X_2
8	0.96	0.93	83	17
15	0.80	0.63	67	33

X_1 = α at 8 days in 8 mmho/cm
X_2 = LAR in control
SPRC = standard partial regression coefficient.

uptake on evapotranspiration flow. Therefore, the contributions of LAR in sodium uptake increases in highly salinized condition. Thus differences in salt tolerance among cultivars are related chiefly with shoot Na content which in turn is affected mainly by Na selectivity in roots and LAR.

To examine whether LAR and root selectivity in a non-salinized condition can be used as screening criteria, a multiple linear regression analysis on LAR, root selectivity under controlled conditions and shoot Na content under salinized conditions during 8 days treatment was conducted. The values showing root selectivity (α) in the control could not be obtained; instead, α values in 8 mmho/cm after 8 days treatment were used because α values in this condition may be close to that in the control. The contribution of LAR to shoot Na content under low salinization condition was 17% (Table 6); however, it increased to 33% in 15 mmho/cm. The contribution therefore of LAR was equally important to that of root selectivity in the highly salinized condition where root selectivity might have decreased due to root deterioration. These results also support the observation that the contribution of each factor to salt tolerance varied with salinization concentration as shown by the differences in salt tolerance at different salinization conditions (Table 3). Generally, cultivars/lines such as KRI-24, IR54 and Bg34–8 have stronger root selectivity against Na similar to tolerant cultivars; because of this, growth in low salinized condition was also higher and similar to the tolerant materials (Table 3). They are often classified as salt-tolerant cultivars in low salinized condition. In highly saline conditions, however, which are more practical conditions for screening, these cultivars/lines could not be considered salt-tolerant as observed in this experiment (Table 3). This is because they have higher LAR than other tolerant cultivars/lines which promotes higher evapotranspiration resulting in higher shoot Na concentration in the highly salinized condition.

Improvement of salt tolerance of the so-called high-yielding dwarf cultivars is urgently needed in breeding programs. These dwarf IR cultivars usually have high LAR (*i.e.*, the incorporation of dwarf genes generally causes reduced growth rate; however, they maintain higher growth rate by expanding the leaf area) while salt-tolerant cultivars like Pokkali, Nona Bokra, and Getu have low LAR. Instead of a greater leaf area, the latter have thicker leaves and higher photosynthetic efficiency per leaf area when grown under non salinized growing conditions. Therefore dwarf cultivars cannot become salt-tolerant unless lower LAR is incorporated. In this aspect, lines derived from anther culture may be short and salt-tolerant in field conditions (F J Zapata, pers. comm.). In this experiment, two of them were highly tolerant (Table 3) and most of them had relatively lower LAR. This also emphasizes the importance of LAR for salt tolerance screening in rice. Iwaki and Ota (1953) reported that salt tolerance varies with growth stage. It is well known that root activity of rice measured by respiration rate decreases rapidly with growth stage (Yamaguchi, 1978). Therefore higher contribution of LAR to salt tolerance in later growth stage can be expected. It is worthwhile to know the change in contribution of LAR and root selectivity to salt tolerance with the change in growth stage.

Na transport to each organ

When Na is absorbed into the plant through the root it is diffused to the sheath and then to the leaf. Therefore sheath Na concentration increases first before leaf Na concentration starts to increase (Fig. 8). Leaf Na concentration will continue to increase to a level similar to that of sheath Na concentration until the plant dies. When sheath Na concentration is lower, leaf Na content becomes almost proportional to the sheath Na content in most cultivars/lines. Leaf Na concentration was almost 1/4 that of sheath Na concentration in all cultivars/lines in the 8 mmho/cm treatment. Little differences in compartmentation of Na ion between sheath and leaf

among cultivars/lines were observed except in BR4-10. However, when sheath Na concentration was increased upon treatment with 15 mmho/cm saline solution, leaf Na concentration increased hyperbolically with an increase in sheath Na content (Fig. 8). Clear differences among cultivars/lines were observed in this relationship depending on leaf weight during the short salinization duration. Cultivars/lines with less leaf dry weight showed an increase in leaf Na content at lower sheath Na content; cultivars/lines with greater leaf weight showed a slower increase in leaf Na concentration after the sheath Na content became higher. This can be explained by the fact that the leaf is the end point of diffusion of Na ion — when the volume of leaf is smaller, leaf Na concentration increases at a faster rate when sheath Na content is low. With a long period of salinization using highly saline solution, leaf Na concentration increases until it is in equilibrium with high sheath Na concentration, a dose almost lethal to the tissue. Under these conditions, the leaf Na concentration shows little correlation with leaf weight. In a prolonged and highly salinized condition higher leaf weights due to seedling vigor may have little effect on salt tolerance although they affect growth in the low salinization condition.

From the observation of tissue death in each organ, leaf death always proceeded from the older leaf to the younger leaf; the roots attached to the internode with dead leaf and sheath appeared to be translucent and yellowish while the roots attached to the internode connected to the living leaf were white and healthy. Moreover, great difference in Na concentration among individual leaves were reported (Yeo and Flowers, 1982). These indicate that the compartmentation of the Na ion among leaves may chiefly be regulated with Na selectivity of roots generated from the same internode and that the movement of the Na ion through the node is limited. Lower sodium uptake of the topmost leaf may be due to the fact that roots from the internode from which the flag leaf generates are not developing. Roots start to generate from the third internode downward the internode which generates the topmost leaf (Kawata *et al.* 1963). Differences

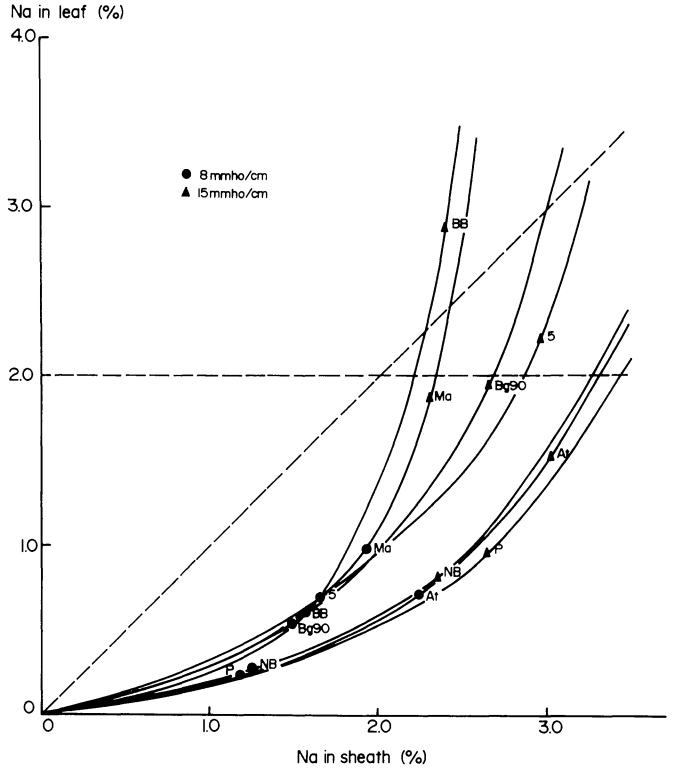

Fig. 8. Increase in leaf Na relative to sheath Na concentration after 8 days treatment.

in the transport process of Na ions and differentials in leaf Na concentration within a plant may also be some of the factors responsible for differential response to salinization among cultivars/lines.

Tissue tolerance

One of the major causes of differences in growth reduction among cultivars/lines during salinization was attributed to tissue tolerance (Yeo and Flowers, 1983). NAR and LAR at a certain leaf Na content would be the best criteria to evaluate the tissue tolerance to growth reduction in the salinized condition. When differences in NAR at the relatively high leaf Na content of 2.5% among cultivars/lines is evaluated, those belonging to group I in Figure 9 showed higher NAR value. The ability of these materials to maintain high photosynthetic activity in the salinized condition was high. Most salt-tolerant cultivars/lines were included in this group. However, when LAR in 2.5% leaf Na was evaluated, those belonging to group I in the former analysis did not show high LAR (Fig. 10). Instead, cultivars like IR8, IR54 and IR5 were included in Group 1 (slow LAR-reducing). Tissue tolerance for leaf area maintenance of these materials under the saline condition was high. Tsuchiya *et al.* (1987b) reported that the dark respiration of shoot of IR28 (susceptible variety) was maintained at higher rate

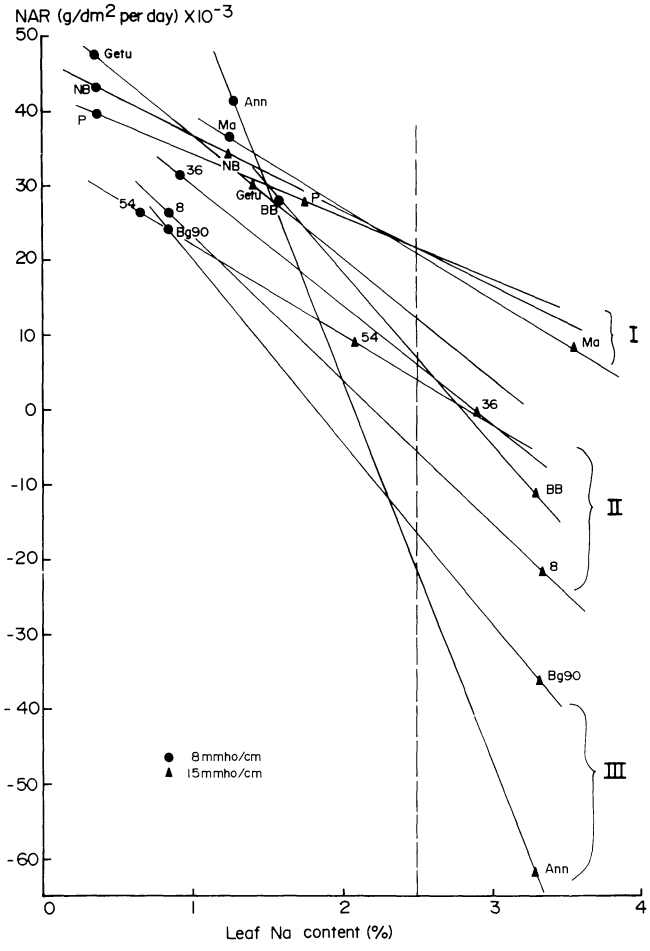

Fig. 9. Effect of leaf Na content on NAR from 8–15 days after salinization with two salt concentration. I = Cultivars P, NB, BR, AC19, AC30, Ma, At. II = Cultivars Getu, 4595, M242, 54, 36, BB, 8, AC26, AC33, ADT. III = Cultivars KR, 5, Bg90, 50, Ann, Bg367. For those cultivars belonging to Group I, LAR at 2.5% leaf Na is high, hence tolerance for maintaining ability of photosynthetic activity is high.

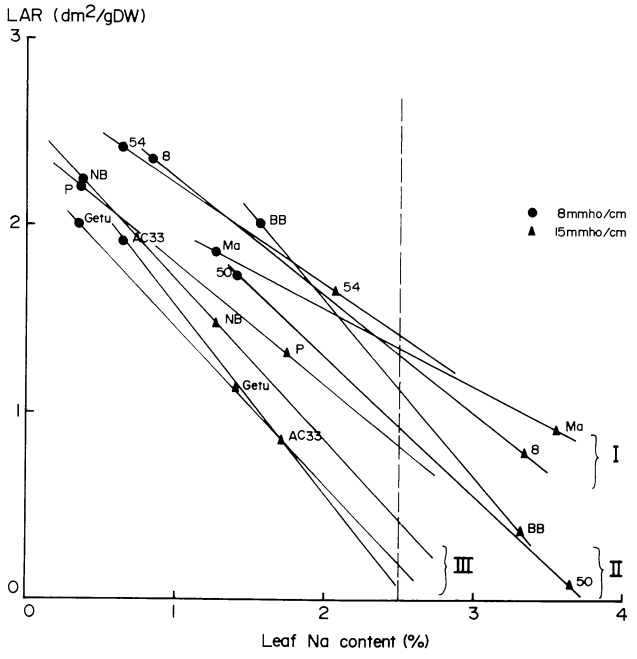

Fig. 10. Effect of leaf Na content on LAR from 8–15 days after salination with two salt concentrations. I = Cultivars Ma, 8, 54, BR, 5 (LAR at 2.5% leaf Na is high, i.e., tissue tolerance is high). II = Cultivars AC30, BB, AC26, Bg90, P, 50, Ann, Bg367, KR, AC19 (LAR at 2.5% leaf Na is intermediate). III = Cultivars ADT, Getu, 4595, AC33, Bg34, M242, 36, NB (LAR at 2.5% leaf Na is low).

under salinized condition than that of Pokkali. Ability to maintain higher dark respiration in the salinized condition would be a measure of high tissue tolerance. Higher transpiration of IR36 in 8 mmho/cm in this experiment also may support the higher tissue tolerance of some IR cultivars in relatively low salinized conditions and would be the cause for slow LAR-reducing characteristics. However, ability to maintain high LAR due to higher tissue tolerance may cause increased Na uptake due to maintenance of higher evapotranspirational area. Thus, materials with the slow LAR-reducing type and higher tissue tolerance for maintenance under salinized conditions would be generally salt-susceptible (Fig.10). Tolerant materials generally do not belong to the slow LAR reducing group; fast LAR-reducing characteristics in high Na content would be an adaptation mechanism to high salinization.

These results indicate that tissue tolerance to salinity varies with evaluation criteria for tissue tolerance and that evaluation dependent on LAR and NAR often complement each other, *i.e.* the materials with higher tissue tolerance for maintenance of photosynthetic activity per unit leaf area

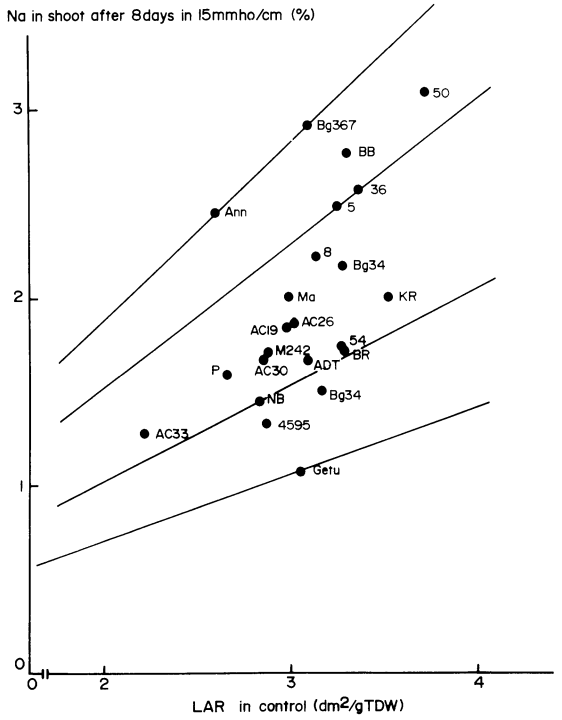

Fig. 11. Relationship between Na concentration in the shoot after 8 days treatment with NaCl at 15 mmho/cm and LAR in the control. Cultivars falling on the same line have similar root selectivity against Na ion.

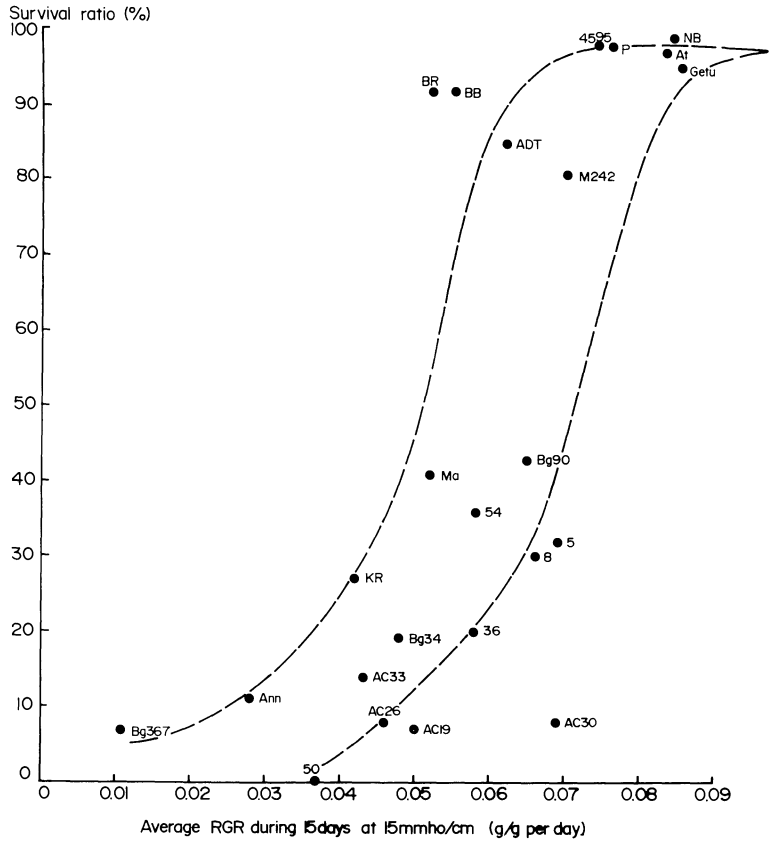

Fig. 12. Relationship between survival ratio and average RGR during 15 days of treatment with 15 mmho/cm salinized solution.

accompanies lower tissue tolerance for leaf area maintenance. Therefore tissue tolerance evaluated by RGR will show less variability. Higher tissue tolerance only for maintaining leaf area or leaf tissue will be inversely related with salt tolerance in whole plant basis and can not be a good criterion for salt tolerance screening in highly salinized condition. This was also supported by the observation that regenerated plants from cell cultures with high tissue tolerance were not always tolerant in the individual plant level (Zapata and Abrigo, 1986).

Screening procedure for high salt tolerance

Significant variations in salt tolerance were observed among cultivars/lines, and tolerance varied with level of salinization even within a cultivar. However, agronomically, tolerance to highly saline condition is more important. Therefore the practical target of screening would be to screen for tolerant materials similar to the most tolerant cultivars (Pokkali and Nona Bokra) in highly saline conditons.

The results mentioned in the preceding sections strongly indicate that root selectivity against the Na ion and LAR are the major characteristics to investigate when developing salt tolerance in individual rice plants under highly saline conditions. Based on these characters screening of salt-tolerant materials can be improved thus: First, screening can be done for lower LAR than that of control tolerant cultivar, not necessarily involving treatment with salinized solution. LAR often relates closely with specific leaf area (SLA: leaf area per unit leaf weight) therefore, SLA can also be used as screening criterion. In this step, materials with poor root selectivity against Na, like Annapurna (Fig. 11), will be retained even though their LAR is lower. Most of the materials with poor tolerance in highly salinized condition, however, can be eliminated. For the small number of materials

remaining, a follow-up screening for root selectivity can be made by observing the survival ratio. Survival ratio of genetically purified materials is related to average RGR during treatment (Fig. 12). This screening should be done at salinization conditions higher than 15 mmho/cm because evaluation of salt tolerance at lower ECs often gives results different from those at high salinity levels. Besides, in conditions prevailing in the actual saline fields, salinity levels fluctuate remarkably so that potentially higher salt tolerance to high salinity level is required. Efficiency in screening of salt-tolerant materials can greatly be improved by the proposed method.

Acknowledgements

The authors are grateful to Dr D Senadhira for valuable suggestions and for providing seeds. We also acknowledge Dr F J Zapata for supplying us seeds of F_1 anther culture materials.

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PLSO PN 186

A method to estimate the prospect of specific breeding for nutrient efficiency

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Key words: genetic correlation, genotype × nitrogen interaction, heritability, low-input-varieties, nitrogen-efficiency, response to selection, *Triticum aestivum* L., winter wheat

Abstract

A biometrical method is described to judge the need of a specific breeding program for cultivars adapted to suboptimal nutrient conditions. Statistical and genetical parameters such as genotype × nutrient interaction, heritability, genetic correlations, expected response to selection are estimated.

The biometrical method is applied to the data of a field experiment with 50 winter wheat (*Triticum aestivum*) genotypes tested at 3 locations and treated with 3 nitrogen levels (low, medium, high). Here the first year's results of the experiment are presented. Yield, yield components, straw length, protein content and protein yield were measured.

The genotype × nitrogen interaction was mostly significant with $p = 0.05$ to $p = 0.01$) when comparing the low with the high nitrogen level. With increasing nitrogen supply the heritability is rising. The genetic correlations between the genotypes tested under low *versus* high nitrogen level were distinctly lower than 1 in almost all characters. The gain of selection for high performance under low nitrogen supply was generally higher in the direct selection (under low nitrogen conditions) than in the indirect selection (under high nitrogen conditions). In most cases the indirect selection under high nitrogen supply was more efficient than direct selection under medium supply.

The results show that a special breeding of wheat cultivars for low nitrogen conditions may be successful.

Introduction

The intensity of agricultural production in central Europe has increased during the past 20 years. Plant breeding has made available cultivars which are adapted to such a high-input system (lodging resistance, tolerance to herbicides). But this modern crop production results in environmental problems (nitrate in ground water, pesticide contamination), problems in plant cultivation and a hardly financeable surplus production.

New laws are likely to emerge which will restrict fertilizer use (nitrogen particularly) and pesticide applications in certain regions so as to protect ground water. In such 'ground water protected areas' continued agricultural production will only be possible under low-input conditions.

In Baden-Württemberg a maximum Nmin-limit has already been fixed for these areas. Other restrictions will follow.

Plant breeding should be prepared for this development because it takes many years to develop new, adapted cultivars. Breeding of cultivars for year 2000 has started already.

For some years resistance breeding has been very intensive in cereals, especially against fungi diseases such as mildew and rusts. But so far only a few activities with selection for nutrient efficiency have been initiated. In breeding it is important to acquire information about the genetic variation that is useful for a special nutrient efficiency program. According to this problem only a few theoretical

approaches and practical experiments have been reported in literature. Muruli and Paulsen (1981) found in maize (*Zea mays* L.) that a synthetic—selected in an environment with high N-fertilization—showed a lower yield in an environment with low-N-supply than the non-selected control population. With direct selection under low N-supply, on the other hand, a positive response to selection could be realized.

Seetharama *et al.* (1984) carried out a similar experiment in an open-pollinating population of sorghum [*Sorghum bicolor* (L.) Moench]. The authors concluded that it will be sufficient to test the low-input qualification of breeding material in advanced generations.

The rice (*Oryza sativa* L.) researches of the IRRI (International Rice Research Institute—Philippines) carried out successful selection programs to improve P and Zn efficiency (IRRI Annual Reports, 1974–83; Mahadevappa *et al.*, 1979; Ponnamperuma, 1977; Ponnamperuma and Ikehashi, 1977). One of the selected lines—IR42 proved to be additionally N-efficient. Old and new cereal cultivars have been compared under field conditions with varying production intensities (for instance: Bachthaler and Pommer, 1980 in barley and oats: Castleberry *et al.*, 1984 in maize; Karpenstein *et al.*, 1986 and Austin *et al.*, 1980 in wheat). Generally the new cultivars proved to be superior even under low-input conditions, but often to a low extent than under high-input conditions. In summary, the results give little evidence on specific ways to select from modern breeding material cultivars adpated to low-input conditions.

In plant breeding there are generally different steps to realize new breeding objectives (see Figure

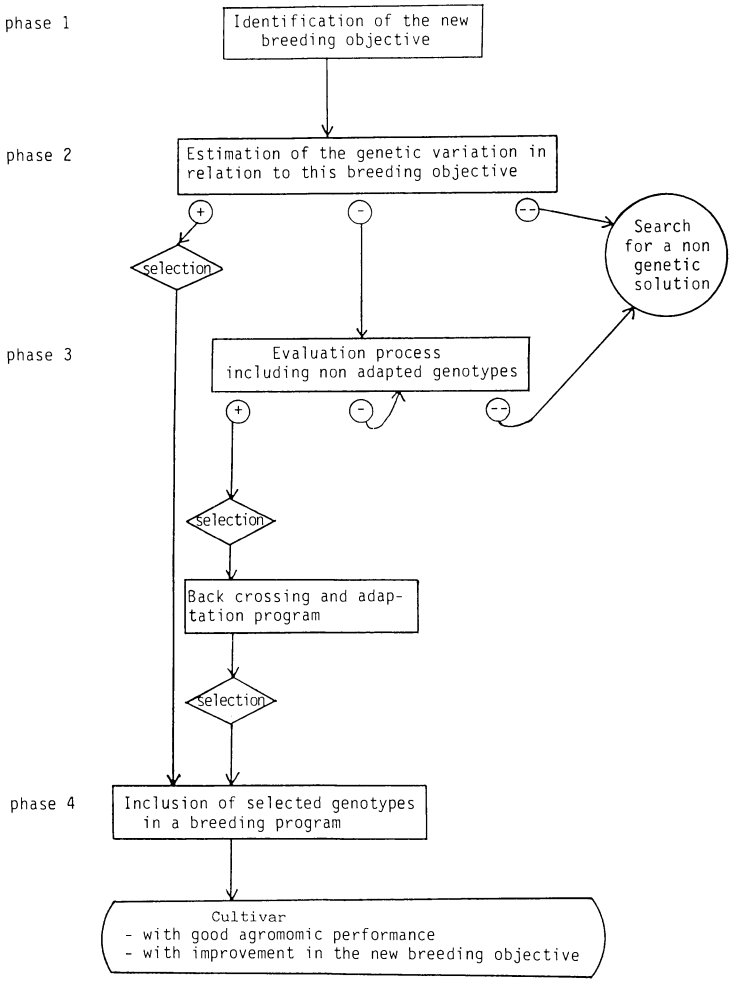

Fig. 1. Realization of new breeding objectives.

1). After identification of a new breeding objective (phase 1) the genetic variation for the characters involved is estimated (phase 2). If there is not enough genetic variation in the adapted breeding material, it will be necessary to include also non-adapted genotypes in the evaluation process (phase 3). The identified genetic variation can be used in a breeding program to select cultivars with a good common agronomic performance combined with improvements in new breeding objective (phase 4). In following as breeding goal the development of winter wheat cultivars with high agronomic performance under N-fertilizer restrictions is considered.

We are presently in phase 2.

Methods

Description of the experiment

Fifty adapted winter wheat genotypes were tested for the first time in 1986/87 under 3 N-fertilizer levels (1 — low 0–40 kg N ha^{-1}, 2 — medium 70–110 kg N ha^{-1}, 3 — high 170–200 kg N ha^{-1}) each with 3 replications in 5 m^3 plots. Only released cultivars and breeding lines were used which are adapted to the Central European climate in order to exclude bias caused by differences in adaptation. The trial was planted at 3 locations: Wetze near Göttingen — heavy soil, 700 mm precipitation; Seligenstadt near Würzburg — heavy soil, 640 mm precipitation; Klausheide, Emsland — sandy soil, 650 mm precipitation.

Fungicides were applied for the whole trial, but no growth regulator was used.

Following characters were investigated in the experiment: Yield and yield components, straw length, kernel protein content (analyzed by NIR-infralyzer) and protein yield.

Biometrical basis

The main objective is to compare the responses to direct selection for high performance under low-input (N-)conditions ($G_{(LI,LI)}$, see Table 1) and indirect selection under high-input (N-)conditions ($G_{(LI,HI)}$, Table 1).

Response to selection is commonly predicted with the following formula:

$$G_{(T,C)} = i \cdot \sigma_T \cdot r_{(T,C')} \cdot h_c$$

with:

T = objective of selection

C = selection criterion

C′ = selection criterion without error effects

$G_{(T,C)}$ = response to selection in T with selection carried out in C.

i = intensity of selection — scheduled factor dependent on the number of selected genotypes.

σ_T, σ_C, $\sigma_{C'}$ = standard deviation in T, C and C′ respectively.

$r_{(T,C')}$ = correlation between T and C′ = 'genetic' correlation between objective and criterion.

$h_C = \sqrt{\frac{\sigma^2_{C'}}{\sigma^2_C}}$ = square root of the heritability of the selection criterion.
= measure of the genetic portion of the phenotypic variation.

Table 1 shows the equation for the response to selection for direct low-input selection (objective = criterion = low-input) and indirect low-input selection (objective = low-input, criterion = high-input). The quotient of the two formulae (U) is a measure of the relative superiority of direct *versus* indirect selection. U depends only on the relative size of the low-input versus high-input square root of heritability and on the reciprocal value of the genetic correlation between low-input and high-input. The formulae for estimation of direct and indirect response to selection, heritabilities and genetic correlations are given in Table 2. In the present experiment the model for a mean value over replications and locations (X_{ij}) can be described as follows:

Table 1. Response to selection — $G_{(T,C)}$ for direct and indirect selection

1. Direct selection for low-input (LI)

$$\frac{G_{LI,LI} = i \cdot \sigma_{LI} \cdot h_{LI}}{\text{objective} = \text{criterion} = LI} \qquad r_g(LI, LI) = 1$$

2. Indirect selection for low-input under high-input (HI)

$$\frac{G_{LI,HI} = i \cdot \sigma_{LI} \cdot h_{HI} \cdot r_g(LI, HI)}{\text{objective} = LI \quad \text{criterion} = HI}$$

3. Relative superiority of direct versus indirect selection U

$$U = \frac{G_{(LI,LI)}}{G_{(LI,HI)}} = \frac{i \cdot \sigma_{LI} \cdot h_{LI}}{i \cdot \sigma_{LI} \cdot h_{HI} \cdot r_{g(LI,HI)}} = \frac{h_{LI}}{h_{HI}} \cdot \frac{1}{r_{g(LI,HI)}}$$

where r_g = genetic correlation
definitions of the other parameters see text.

Table 2. Estimation formulae for direct and indirect response to selection, heritability and genetic correlation

A.) *Modell:*

$X_{ij} = \mu + t_i + n_j + tn_{ij} + \bar{e}_{ij}$

where: μ = general mean
t_i = genetic effect of variety i; i = 1, ..., T
n_j = effect of N-supply level j; j = 1, ..., U
tn_{ij} = interaction effect of variety i on N-supply level j

B.) *Estimation formulae for C = level 3 and T = level 1*

$$\hat{G}_{(T,C)} = i \cdot \hat{\sigma}_T \cdot \hat{h}_C \cdot \hat{r}_{g(T,C')}$$

$$\hat{h}_C = \sqrt{\frac{\hat{\sigma}^2_C}{\hat{\sigma}^2 c}}$$

$$\hat{r}_{g(T,C')} = \frac{\hat{cov}(T,C')}{\sqrt{\hat{\sigma}^2_T \cdot \hat{\sigma}^2_{C'}}}$$

where: $\hat{\sigma}^2_T = \frac{1}{T-1} \sum_i (x_{i1} - x_{.1})^2 - \sigma^2_{\bar{e}_1}$

$$\hat{\sigma}^2_C = \frac{1}{T-1} \sum_i (x_{i3} - x_{.3})^2$$

$$\sigma^2_{C'} = \hat{\sigma}^2_C - \sigma^2_{\bar{e}_3}$$

$$\hat{cov}(T,C') = \frac{1}{T-1} \left(\sum_i (x_{ij} - x_{.1})(x_{i3} - x_{.3})\right)$$

with: $X_{.j} = \frac{1}{U} \sum_i X_{ij}$

$\sigma^2_{\bar{e}_j}$ = error variance of level j, calculated by separate analysis of variance

$$X_{ij} = \mu + t_i + n_j + tn_{ij} + \bar{e}_{ij}$$

(definition of the different effects see Table 2). In this situation the significance of the variation of the genotype × N-supply interaction effects (tn_{ij}) is important.

$tn = 0$ means that the rank of all genotypes is the same under low-input and high-input conditions.

$tn > 0$ means that the ranking of the genotypes is different in the low-input and the high-input environment (see Figure 2).

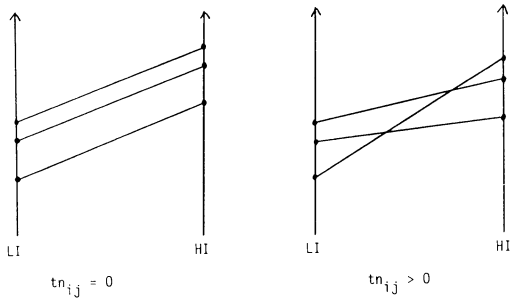

Fig. 2. Scheme of genotype × N-supply interaction — tn_{ij}.

Results and discussion

Mean values

Data in Figure 3 show the mean values at the different nitrogen levels. The grain yield increases with higher N-supply. The larger number of

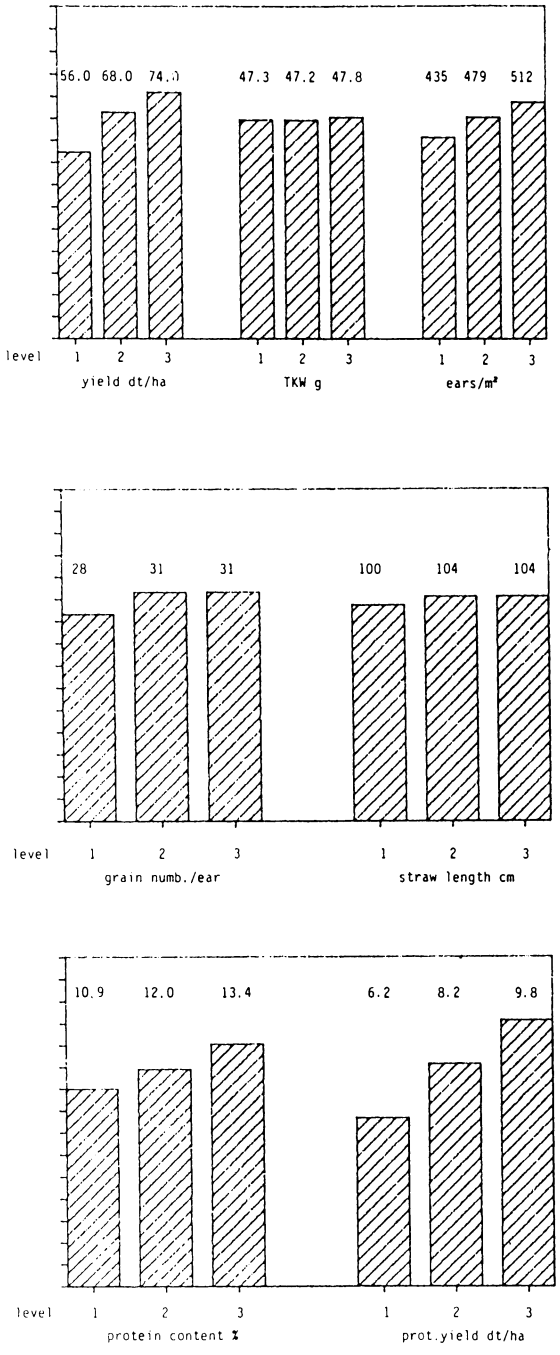

Fig. 3. Mean values of the three nitrogen levels.

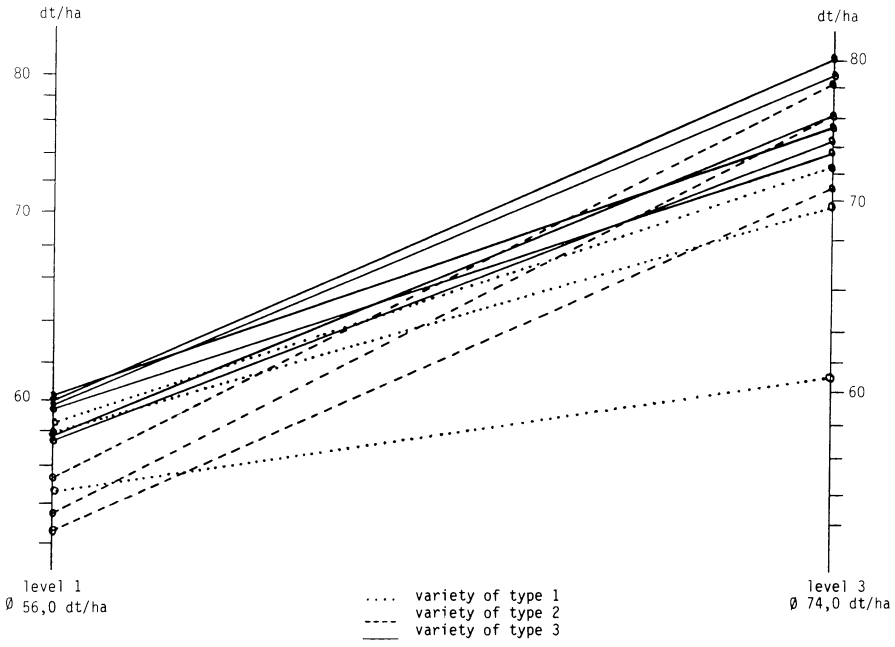

Fig. 4. G × N supply interaction for yield of 12 cultivars (Comparison of level 1 and 3).

ears m^{-2} is the primary cause. The grain number/ear is rising only between the low and the medium level and the thousand kernel weight between the medium and the high level. The protein content also increases with N-supply. Consequently the protein yield is evidently rising with fertilization. The straw length increases by about 4 cm between the low and the medium level because nitrogen generally enhances vegetative growth.

Genotype × N-supply-interaction

Considering level 1 as low-input and level 3 as high-input the genotype × N-supply interaction is significant ($\alpha = 0.05$ to $\alpha = 0.01$) for all characters besides thousand kernel weight. The comparison of level 2 as low-input with level 3 as high-input, hardly shows significance. The genotype × N-supply interaction is significant only for protein yield.

In Fig. 4 twelve representative genotypes were selected to demonstrate the genotype × N-supply yield interaction (comparison of level 1 and 3). The varieties tend to fall into three groups:

Type 1: cultivars with a relative low yield increase.
= relative better performance under low-input conditions.

Type 2: cultivars with a relative marked yield increase with higher N-supply.
= relative better performance under high-input conditions.

Type 3: intermediate cultivars.

Heritabilities

Data in Table 3 show the heritabilities at the different N-levels. In general heritability is rising with N-supply. This response is less evident in highly heritable characters such as thousand kernel weight and straw length.

Similar results have been published for example by Pfeiffer (1983) with spring wheat, Rao *et al.* (1981) with sorghum and Allen *et al.* (1978) with

Table 3. Heritabilities (h^2) of the different nitrogen levels

Character	Low level 1	Medium level 2	High level 3
Yield	0.56	0.61	0.65
Thousand kernel weight	0.86	0.90	0.90
Ears/m^2	0.55	0.40	0.71
Grain number/ear	0.48	0.53	0.75
Straw length	0.90	0.95	0.90
Protein content	0.81	0.84	0.88
Protein yield	0.22	0.53	0.70

wheat. They found that more productive environments generally show a higher heritability for grain yield than less productive environments.

Genetic correlations

The genetic correlation between low-input and high-input performance is very important for indirect selection. Data in Table 4 show genetic correlations between high-input = level 3 and low-input = level 1 or 2, respectively. Because the genetic correlation increases with decreasing genotype × N-supply interaction, it is evident that the correlations of the highly heritable characters thousand kernel weight and straw length are close to 1. Comparing level 1 with level 3 the correlation of all other characters are distinctly lower than 1 which means that in level 1 other genotypes than in level 3 would be selected. Comparing level 2 with level 3 all genetic correlations are 1 or close to 1 because the genotype × N-supply interactions are very low (see above). Due to high standard errors in estimation, some genetic correlations are partly over-estimated. (Estimates higher than 1 are written as 1 in Table 4).

Response to selection

The ratio of direct to indirect response to selection (U) and its components — square root of heritability and genetic correlation — are given in Figure 5. Direct selection is more efficient than indirect selection especially when comparing level 1 and 3. This is true in spite of the generally lower low-input heritability because the genetic correlation is often lower than the quotient h_{LI}/h_{HI}. There is only a small advantage for direct selection in the highly heritable characters (thousand kernel weight, straw length). The superiority of direct selection is more evident in the low heritable characters yield (+16%), ears m^{-2} (+181%), grain number/ear (+34%) and protein content (+42%). This is particularly important because this advantage would accumulate for several years during breeding programs. The protein yield differs from the other characters. The indirect selection is more efficient than the direct selection (level 1) inspite of the low genetic correlation because h_{LI} is extremely low.

Table 4. Genetic correlations between low-input and high-input — $r_{g(LI,HI)}$

Character	LI = level 1 HI = level 3 $r_{g(1,3)}$	LI = level 2 HI = level 3 $r_{g(2,3)}$
Yield	0.80	0.99
Thousand kernel weight	0.97	1
Ears/m^2	0.31	1
Grain number/ear	0.60	1
Straw length	0.92	1
Protein content	0.68	0.95
Protein yield	0.62	1

Comparing level 2 and 3 the ratio U depends almost exclusively on the heritabilities because the genetic correlation has values of nearly 1. Therefore indirect selection is equally or more efficient in almost all characters.

It needs to be noted that these are only results of one year and for a conclusive interpretation data of more years are required. Besides the greater part of the material is preselected under conventional conditions which might result in a loss of genetic variation (for N-efficiency). Nevertheless the results indicate that there may be enough genetic variation useful for a special N-efficiency breeding program, especially for the most important characters yield and its components (besides thousand kernel weight) and protein content. But if only a moderate N-restriction (level 2 instead of level 3) is implemented, which might be realistic in the near future, a special N-efficiency selection program will not be advantageous. However the interaction components and genetic correlations are best estimated at relatively different levels (level 1 *versus* level 3 in contrast to level 2 *versus* level 3). By selecting for higher N-efficiency, the genetic correlations between LI and HI would probably become smaller in the longterm. On the one hand, the allels — positive under both cultivation conditions — become fixed with progressive selection and variation of special low-input and high-input allels remains constant. Beet offers a practical example for such a development. Selecting for higher sugar content in fodder-beet sugar yield would also rise because of the positive genetic correlation between the two characters. With increasing sugar content the genetic correlation becomes smaller and eventually negative. Selection for

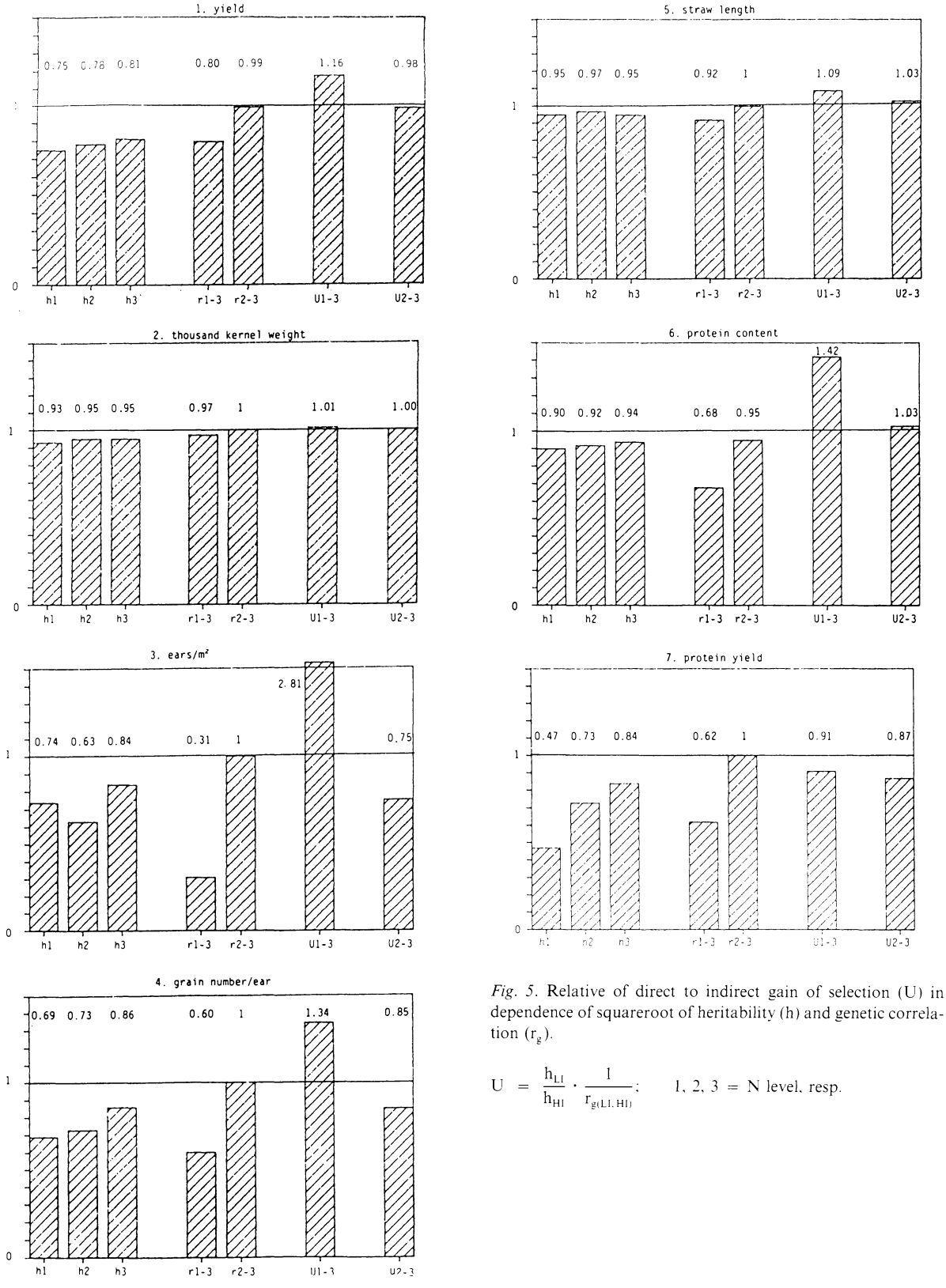

Fig. 5. Relative of direct to indirect gain of selection (U) in dependence of squareroot of heritability (h) and genetic correlation (r_g).

$$U = \frac{h_{LI}}{h_{HI}} \cdot \frac{1}{r_{g(LI,HI)}}; \qquad 1, 2, 3 = \text{N level, resp.}$$

higher sugar content in sugar-beet is a selection for lower sugar yield.

Nevertheless the genetic variation in a breeding program with aimed crosses and selection in segregating generations could be higher than could be expected from the present results.

In a low-input breeding program the N-efficiency has to be combined with fungi disease resistance without a deterioration in other important characters. In the longterm the introduction of non adapted material could be envisaged for such a breeding program so as to widen the special variation.

Acknowledgements

The authors thank Prof H H Geiger, Hohenheim for the intensive support and the stimulating discussions, Dr R von Brook and Miss A Kunert, Einbeck for the critical reading of the manuscript and Dr K Brunckhorst, Wetze for extensive advice relating to field experimentation.

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PLSO PN 187
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Grain yield and quality characters of genotypes in F_5 generation under low and high nitrogen input

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Key words: breeding, crude protein, grain hardness, nitrogen input, quality characters, sedimentation value, *Triticum aestivum* L., yield performance

Abstract

Conventional wheat (*Triticum aestivum* L.) breeding program, based on intensive selections for disease resistance and on screening for yield and quality in early segregating generations, has been completed using orthogonal tests and a weighted combined selection of genotypes under low and high nitrogen input starting in F_5 generation.

Absolute values for grain yield, crude protein and sedimentation value in the 'low nitrogen' location were lower than those found in the 'high nitrogen' environment. A significant correlation between the relative yields in the two nitrogen environments indicates, that in general, selection on a high nitrogen level results in genotypes with a high relative performance also under low nitrogen conditions. Beside a relatively large percentage of genotypes with similar performance in the tested environments ('combination' types), many F_5 strains performed only in the sand soil with low nitrogen ('low input' types) or only in the productive soil with high nitrogen input ('high input' types) above the average of the high yielding check cultivar. The relative classification of the material for quality is very similar in both environments. Differences in quality characters between the locations for the same genotype give a first point on genotypic interaction to nitrogen input also for crude protein in grain, which can be partly explained by differences in yield performance.

Introduction

The increase of mean yields during the last 10 years in FRG has been linear and amounted to 1.8 q/ha annually; the increase was due to the success of breeding programs and an increase of nitrogen fertilization in combination with an intensive application of fungicides and growth regulators. Political, ecological and economical demands may initiate changes away from the high intensity production levels to low input situations such as a reduced input of nitrogen. The breeder should provide cultivars with high relative yield performance, independently from nitrogen input. In cooperation with Prof. Röbbelen, Institute of Agronomy and Plant Breeding of the University Göttingen, a long term project has been started to investigate the necessity and possibility of breeding wheat for improved nitrogen utilization.

The term 'nitrogen utilization' includes two parameters: *nitrogen uptake*: biomass production per unit of plant available nitrogen and *nitrogen efficiency (nitrogen use)*: grain and protein production per unit of nitrogen uptake.

For both parameters, many complex factors are responsible, which can not be directly included in selection programs, since suitable screening methods are currently not available. Selection for improvement of nitrogen utilization could thus be possible, if the following conditions are fulfilled: 1. large numbers of genotypes in early generations with a wide genetical range can be screened; 2. radically different levels of available nitrogen under field conditions can be granted and 3. year effects can be taken into consideration through continuous selection.

In this first communication, first years results on

yield and quality performance of genotypes under low and high nitrogen conditions are reported.

Material and methods

Breeding material

In 1986, a total of 967 single plant progenies in F_5 generation and some cultivars have been orthogonally sown in two locations — Warmse and Schöningen — in four trials (Nr. 51–54), each trial including 291 entries — trial 54 241 entries — in one replication using the high performance, wide spread cultivar ARES as check plots for local control; the plot size has been 5.6 sm (Warmse) and 4.5 sm (Schöningen). The single plant progenies (genotypes) represent 97 single and three-way crosses including 104 different parents, so that they cover a very wide genetical and phenotypical range. The single plant progenies have previously undergone a multistage, weighted intensive selection for resistance properties and agronomical characters. They were for the first time selected in the F_4 generation for yield performance and quality characters.

Locations — nitrogen management

Low N-input location Warmse. Warmse is located between Braunschweig and Celle; it is characterized by a sandy soil with a poor N-mineralization ability; the soil has a bonitation of 35 points; the previous crop has been faba beans (*Vicia faba* L.). Nitrogen fertilizer have been given to a total of 100 kg N/ha in four times during growing period.

High N-input location Schöningen. Schöningen is located eastern of Braunschweig at the border to GDR; it is characterized by a high productive loam soil with a bonitation of 85 points; it has a high N-mineralisation ability. The previous crop has been oats (*Avena sativa* L.). 200 kg N/ha were given in four times during growing period.

In both locations no fungicides and no growth regulators were used. Heavy attacks with diseases made possible combined selections for disease resistance properties before harvest, *so that only ca. 40% of the genotypes were orthogonally harvested in both locations.* In Warmse selections could be made against powdery mildew, eye spot and for lodging resistance; in Schöningen selections were combined against powdery mildew, *Puccinia striiformis*, *Puccinia recondita* and ear diseases (*Septoria nodorum*, powdery mildew, *Pseudomonas syringae*).

Quality characters

Samples of selected genotypes in both locations have been investigated for crude protein and grain hardness by means of Near Infrared Reflexion (NIR). Sedimentation values were determined by Zeleny test. Specific sedimentation values were calculated by means of the formula: z = sed. value: (protein % − 5).

Results and discussion

Yield performance

The absolute yield values for the check cultivar Ares (Table 1) indicate as expected big differences between low and high N-location: Schöningen was 20–30% higher than Warmse.

In both locations, the check cultivar has performed over all trials in a comparable level, expressing thus a good uniformity of the trial fields and the yield stability of the check.

The mean relative performance of the harvested F_5 genotypes (100% = check Ares) indicated an

Table 1. Correlations of relative yields of F_5 – genotypes between low (Warmse) and high (Schoeningen) N-input

Character	Trial			
	51	52	53	54
n genotypes	96	100	120	92
Yield (q/ha)				
check ARES Wa	63.3	62.3	64.7	64.7
(n = 30) Schoe	79.7	78.6	79.2	84.8
Rel. yield (%)				
Range Wa	81–128	81–129	86–125	87–121
Range Schoe	84–122	80–124	82–119	88–118
Mean Wa	105	107	107	101
Mean Schoe	101	104	103	103
r	0.359**	0.310**	0.168**	0.324**
r^2	0.13	0.10	0.03	0.10

improvement in the yield potential of this early generation in relation to the check; the variation range of relative yield was between 80–130% and indicated that the breeding potential for yield improvement has not been exhausted.

The correlation coefficients[1] of relative yields between low and high N-environment were significant (Table 1) and indicated that a part of the harvested genotypes had a similar relative performance to the check independent from location; but the values are low and express also an interaction of genotype × N-location (Fig. 1).

Under low and high N-conditions, genotypes showed different relative yield reactions. The genotypes in trial 53 (Fig. 2) cover the whole range of variation of relative yield depending on the location, but also a part of the genotypes was concentrated around the regression line. Both figures demonstrate the possibility to select cultivars with higher performance in both environments (combination types) and also cultivars with special adaptation to one environment (low respectively high input types). To explain the differential reactions in yield performance, intercultivar correlations between yield and several agronomical characters have been calculated for each environment. The results are summarized in Table 4. Differences among the genotypes in straw length and heading date can not generally explain the differences found for yield. Positive intercultivar correlations between yield and 1,000 grain weight showed that large grain cultivars produced higher relative yields in both locations. Significant correlations of relative yield to mildew and eye spot were found in Warmse and to *P. recondita* and ear diseases in Schöningen (Table 4).

For better characterization of possible relationships between relative yield under low and high inputs and specific phenotypical characters, the harvested genotypes have been classified over all trials in 9 yield classes according to the scheme explained in Table 2; for this classification, the differences of relative yield of each genotype between the two locations and the relation of the relative yield of each genotype to the check cultivar have been considered. The means for relative yield and different characteristics of the genotypes in each yield class and for each location are summarized in Table 3. The classes 11, 12, 13 include genotypes (ca. 38%) with identical yield performance under low and high N-input (combination types, Fig. 3). The classes 21, 31, 41 include 161 genotypes (40%) with a higher relative performance under the low input conditions of Warmse (low input types, Fig. 3). The 'high input' classes 22, 32, 42 include 89 genotypes (22%). There was no evidence that the genetic origin of the genotypes in the different classes can be responsible for their different reaction under low and high N-input.

There was also no evidence that special phenotypical features are typical for genotypes of the different yield classes. The genotypes of classes 31 and 41 (relative performance in Warmse clearly better) have an average 1,000 grain weight that is higher than that of the genotypes of the classes 32 and 42, confirming its slight positive correlation to yield shown in Table 4. The genotypes of the best yield class 13 have in common a very good resistance combination to all diseases in both environments. Higher attack with eye spot explains partly the weak performance of the genotypes of the classes 11, 22, 32 and 42 in Warmse in combination with higher average values for lodging. The genotypes with lower performance in Schöningen (class 11, 21, 31 and 41) showed higher average values for *Puccinia recondita* and ear diseases. For the different performance reactions of the genotypes, differences in resistance properties are chiefly responsible. Though, these differences can not fully explain the very big differences of relative yield performance of the genotypes included in the classes 31, 41 and 32, 42.

Quality characters

Crude protein % (N × 5.75). In all trials, the high N-location (Schöningen) had high absolute yields and high levels of protein; the mean difference amounts to ±1% protein (Table 5). The wide range of variation between the genotypes in both locations can be explained mainly by differences in yield performance especially for Warmse since correlations are negative and highly significant (Table 4). The genotypes with lower performance in Warmse (yield class 11, 32 and 42) have a higher

[1] The plots of the check cultivar Ares are not included in the correlations

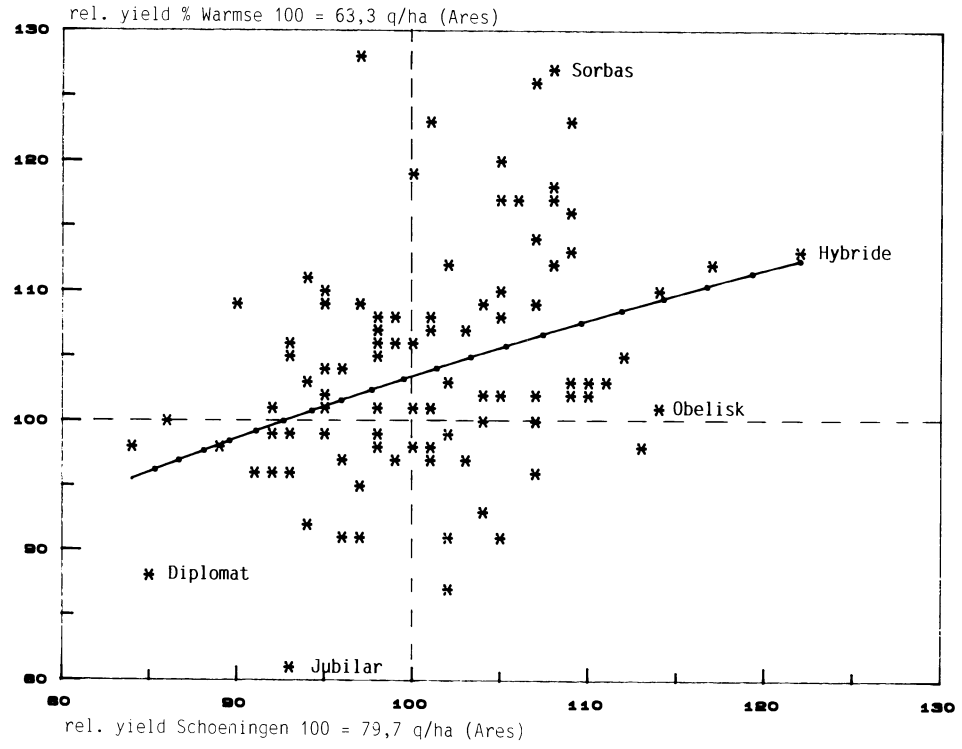

Fig. 1. Relative yields of F_5-genotypes under low (Warmse) and high (Schoeningen) N-input.

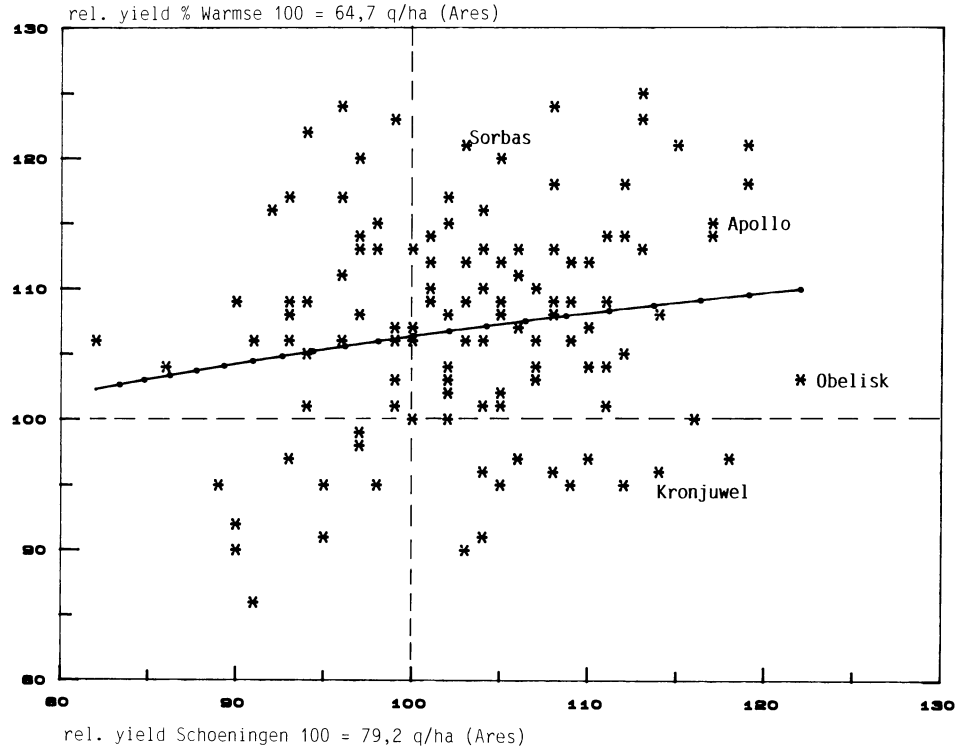

Fig. 2. Relative yields of F_5-genotypes under low (Warmse) and high (Schoeningen) N-input.

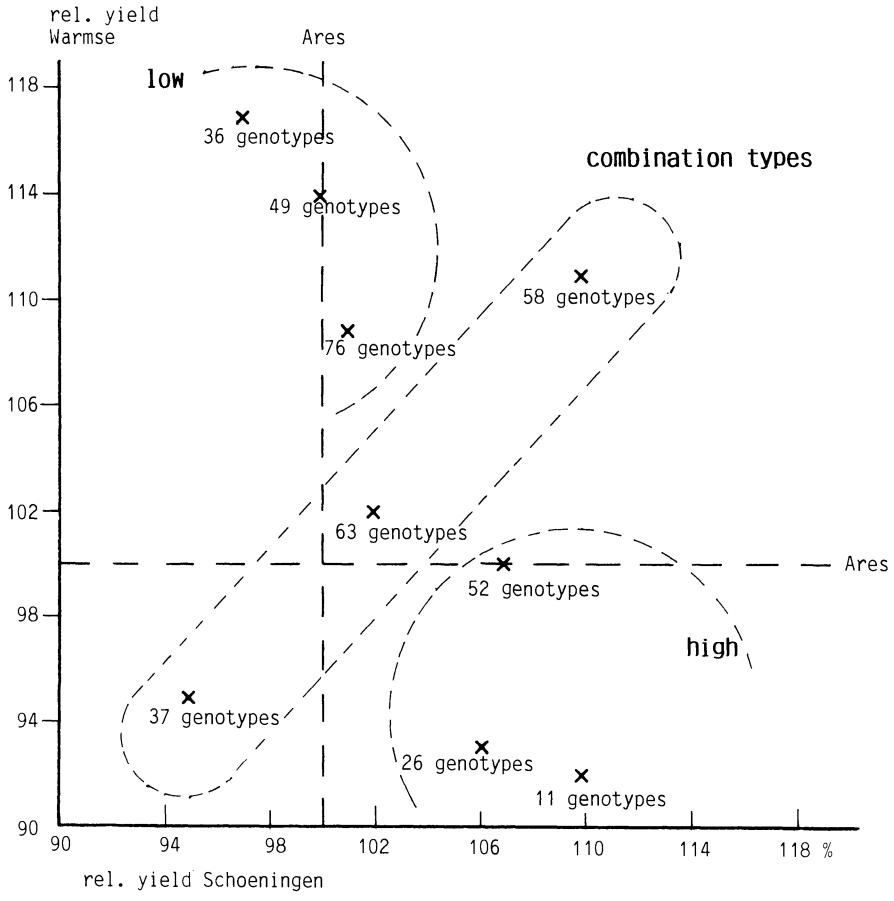

Fig. 3. Means of relative yields and number of F_5-genotypes under low (Warmse) and high (Schöningen) N-input in different yield classes.

Table 2. Scheme of classification of relative yield under low (Warmse) and high (Schoeningen) N-input

Class	Differences between locations (rel. yield %)			
	0–5%	6–10%	11–15%	⩾16%
	1	2	3	4
1	in both locations <98%	Wa > Schoe	Wa > Schoe	Wa > Schoe
2	in both locations 99–105%	Wa < Schloe	Wa < Schoe	Wa < Schoe
3	in both locations ⩾106%	–	–	–

Table 3. Means for relative yield and agronomic characters of F_5-genotypes under low (Warmse) and high (Schoeningen) N-input in the different yield classes

Yield class	Loc.	Geno-types	Rel. yield (%)	Crude Protein (%)	Sed. value	1000 GW	Straw length	Lodging[a]	Mildew[a]	Eye spot[a]	*P. striiformis*[a]	*P. recondita*[a]	Ear diseases[a]
11	Wa	37	95	12.1	31	46.8	105	1.9	3.7	5.1	–	–	–
	Schoe		95	13.0	32	48.4	108	–	3.4	–	1.7	4.6	4.9
12	Wa	63	102	11.8	31	45.3	107	1.6	3.2	4.0	–	–	—
	Schoe		102	12.8	32	46.8	110	–	3.3	–	1.7	4.1	4.5
13	Wa	58	111	11.3	26	45.7	107	1.8	2.8	3.8	–	–	–
	Schoe		110	12.7	28	47.4	109	–	3.2	–	1.4	3.3	4.6
21	Wa	76	109	11.7	29	45.7	107	1.6	3.0	3.3	–	–	–
	Schoe		101	12.6	30	46.0	109	–	3.0	–	1.8	4.4	4.7
22	Wa	52	100	11.8	30	45.2	107	2.0	3.4	5.1	–	–	–
	Schoe		107	12.9	31	47.7	111	–	2.9	–	1.5	3.0	3.9
31	Wa	49	113	11.5	29	47.0	109	1.8	2.9	3.9	–	–	–
	Schoe		100	12.8	32	46.1	111	–	2.9	–	1.6	5.0	4.3
32	Wa	26	93	12.1	34	42.9	108	2.5	3.9	5.1	–	–	–
	Schoe		106	12.9	34	45.6	111	–	3.6	–	1.4	3.5	4.0
41	Wa	36	117	10.9	28	46.7	106	2.0	3.5	3.8	–	–	–
	Schoe		97	12.4	32	45.6	109	–	3.5	–	2.0	5.6	4.8
42	Wa	11	92	11.3	29	44.8	109	2.6	3.8	4.5	–	–	–
	Schoe		110	12.2	29	44.9	113	–	4.0	–	1.5	4.0	3.9

[a] Scores: 1 = no lodging, free of diseases.
9 = full lodging, heavy attack.

Table 4. Correlations of relative yields to agronomic characters of F_5-genotypes under low (Warmse) and high (Schoeningen) N-input

Character		Trial 51		52		53		54	
relative yield (%) to:	loc.	$\bar{x}$	r	$\bar{x}$	r	$\bar{x}$	r	$\bar{x}$	r
1,000 GW (g)	Wa	45.4	0.277*	45.6	0.245*	46.7	0.245*	45.5	0.037 ns
	Schoe	48.0	0.302*(1)	45.0	0.063 ns	47.5	0.101 ns	46.4	0.295*
Straw length (cm)	Wa	107	0.283**	108	−0.045	107	0.078 ns	106	−0.04 ns
	Schoe	108	0.145 ns	111	0.306**(1)	113	−0.115 ns	106	0.09 ns
Heading date	Wa	54	−0.01 ns	55	0.165 ns	53	0.011 ns	55	0.119
	Schoe	53	−0.07 ns	52	−0.067 ns	51	0.329***(1)	55	0.327**(1)
Lodging	Wa	2.0	0.09 ns	1.8	−0.197*	1.2	0.114 ns	2.3	−0.278**
	Schoe	–	–	–	–	–	–	–	–
Mildew	Wa	3.3	−0.217*(1)	3.1	−0.241*	3.1	−0.342***	3.5	−0.284**
	Schoe	4.2	−0.441***	3.1	−0.176+	2.9	0.067 ns	3.0	0.100 ns
P. striiformis	Wa	–	–	–	–	–	–	–	–
	Schoe	1.4	−0.219*	1.5	0.212	1.7	−0.09 ns	1.9	−0.133 ns
P. recondita	Wa	–	–	–	–	–	–	–	–
	Schoe	3.6	−0.407***	5.2	−0.265**	4.7	−0.326(1)	2.9	−0.248*
Eye spot	Wa	5.0	−0.282**(1)	4.5	−0.214*	3.0	−0.322***	4.4	−0.318**
	Schoe	–	–	–	–	–	–	–	–
Ear diseases	Wa	–	–	–	–	–	–	–	–
	Schoe	4.8	−0.195+	4.4	−0.396***	4.6	0.05 ns	4.1	0.251*
Crude protein (%)	Wa	11.7	−0.286+(1)	11.5	−0.370**	11.5	−413**	11.7	−0.404**
	Schoe	12.8	0.02 ns	12.5	−0.02 ns	12.7	−0.075	12.8	0.131 ns

(1) non linear regression.

protein content under the low N-input (Table 4), confirming the negative yield/protein correlation. Although the nitrogen input in Warmse and Schöningen was very different, the tested genotypes show on both locations in their range a good correspondence for protein content, which is highly significant (Table 5) and better correlated than for yield.

Sedimentation value-specific sedimentation value-grain hardness

The wide variation range of these characters on both N-environments expressed the great differences among genotypes; all quality groups are represented. The mean values for sedimentation and grain hardness (Table 5) were slightly higher in Schöningen than in Warmse. The very high correlation coefficients for these characters between high and low-input conditions demonstrated an almost identical behaviour of the genotypes in both locations and confirm further the high heritability of these quality characteristics. These results show that the relative classification of genetic material for baking quality has not been unfavorably influenced by the differential nitrogen inputs.

Continuing investigations, representing 'high input', 'low input' and 'combination' genotypes, are focusing on continuous selection under differential nitrogen levels and evaluation of these special genotypes for different nitrogen utilization patterns.

Acknowledgements

We thank Prof. Röbbelen, Institute of Agronomy and Plant Breeding, University Göttingen, for cooperation and scientific advice.

Table 5. Correlations for indirect quality characters of F_5-genotypes between 'low' (Warmse) and 'high' (Schoeningen) N-input

Quality character	Trial				
	51	52	53	54	range
n genotypes	52	65	80	50	51–54
Crude Prot. (%) (NIR)					
Mean Wa	11.7	11.5	11.5	11.7	9.3–13.0
Mean Schoe	12.8	12.5	12.7	12.8	11.1–13.8
r	0.685***	0.486**	0.617***	0.780***	
r^2	0.47	0.24	0.38	0.63	
Sed. value (Zeleny)					
Mean Wa	31	30	28	25	13–60
Mean Schoe	33	32	30	26	14–68
r	0.911***	0.937***	0.952***	0.671***	
r^2	0.83	0.88	0.91	0.45	
Spec. sed. value $z = \frac{\text{sed. value}}{\text{prot. \%} - 5}$					
Mean Wa	4.7	4.7	4.3	3.9	2.2–8.7
Mean Schoe	4.3	4.3	3.8	3.3	1.9–8.1
r	0.953***	0.963***	0.957***	0.828***	
r^2	0.91	0.93	0.92	0.69	
Grain hardness (NIR)					
Mean Wa	50	51	47	44	34–66
Mean Schoe	53	53	51	46	36–72
r	0.926***	0.911***	0.943***	0.971***	
r^2	0.86	0.83	0.89	0.94	

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 465–473.
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PLSO PN 188

Performance of winter wheat cultivars under reduced nitrogen conditions

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Key words: cultivars, grain nitrogen yield, grain yield, nitrogen input, *Triticum aestivum* L., wheat

Abstract

Fourteen winter wheat (*Triticum aestivum* L.) cultivars with known differences in genetic background, in morphological characters and in yield potential are tested in two locations (loess loam soil and sand soil) under three nitrogen levels: 200 kg N/ha, 100 kg N/ha and no nitrogen fertilization. One year results are presented for grain yield and grain nitrogen yield. Significant differences among nitrogen levels and among cultivars were found for grain yield and grain nitrogen yield. Ranking of cultivars for grain yield and grain nitrogen yield was different. Interactions of nitrogen levels × cultivars were significant for grain yield and grain nitrogen yield; the interaction locations × nitrogen levels × cultivars was significant only for grain nitrogen yield. Nitrogen Use Indices (NUI) showed differences among cultivars but no differences among N-levels.

Introduction

The response of wheat cultivars to increased mineral nitrogen fertilization has been the chief subject of innumerable investigations and field trials all over the world. Breeders are also using cultivar specific reactions to increased nitrogen input as a basis for their consultative services for better management and best performance utilization of their own cultivars. In nitrogen intensive programs investigation of cultivar reactions to reduced nitrogen fertilization might be viewed as an anachronistic development. Nevertheless, if practical genetic improvement of nitrogen utilization is to be accomplished, information on reactions of modern, high performance wheat cultivars under extremely reduced nitrogen conditions is essential.

In this sense, parallel to selections under reduced nitrogen input in two locations, Schoeningen and Warmse (Spanakakis, 1990), an orthogonal two year cultivar trial was started in 1986. In this communication grain yield and grain nitrogen yield of the involved wheat cultivars are presented.

Material and methods

Fourteen winter wheat cultivars (Table 1) have been included in orthogonal trials, sown in two locations — Warmse and Schoeningen — using the split plot design with three replications. Plot size was 5.6 sm (Warmse) and 4.5 sm Schoeningen).

Wheat cultivars

Cultivars cover a wide range of genetical diversity — conventional cultivars, one semidwarf, one hybrid, are characterized by typical differences in agronomical characters, yield structure and quality (Table 1) and represent examples of old (Diplomat — Jubilar), new (Obelisk) and probable future breeding progress (Hybrid).

Locations

Warmse is characterized by a sandy soil with low

Table 1. Description of cultivars used in trials

Cultivars	Breeder	Year of registr.	Quality+ group	Maturity+	Straw+ length	1000+ GW	Tillering+
Apollo	Breun	1984	C 2	5	4	5	5
Ares	Strube	1983	B 4	4	7	6	4
Bernina	Zürich/ Reckenholz	1983	B 4	5	5	5	5
Brimstone	PBI	1983	B 4	5	3	7	5
Diplomat	Firlbeck	1966	A 7	4	6	5	5
Jubilar	Schweiger	1961	B 4	5	7	5	4
Kraka	Pajbergfonden/ Petersen	1982	A 6	6	7	5	6
Kron-juwel	Bayer. Pflanzenzucht	1980	B 4	5	4	4	5
Milan	Lochow	1983	B 5	6	8	8	5
Obelisk	Zelder/Strube	1987	B 4	5	4	5	4
Rektor	Firlbeck	1980	A 9	5	6	4	6
Sorbas	Strube	1985	B 4	6	6	7	5
Astron	Strube	NLT	A 7	6	4	5	5
Hybrid	Nickerson	(NLT)	(B4)	5	5	7	5

+explanation of notes: 1: very early, very short, very low; 9: very late, very long, very high.
quality: C: feed wheat; B: standard baking quality; A: good — very high quality.

organic matter and poor N-mineralisation ability. The soil value is 35 points and the previous crop had been faba beans (*Vicia faba* L.).

Schoeningen is characterized by a high productive loess loam soil with a soil value of 85 points, it has a high N-mineralisation ability. The previous crop had been oats (*Avena sativa* L.).

Management of trials

For each cultivar and location three different treatments with nitrogen fertilizer were used:
N0 — no mineral nitrogen fertilization
N100 — 100 kg N ha^{-1}
N200 — 200 kg N ha^{-1}

Mineral nitrogen (calcium ammonium nitrate) was supplied four times during the growing period as follows:
during tillering (EC 23): N100: Warmse 40 kg N ha^{-1}, Schoeningen 45 kg N ha^{-1}.
N200: both locations 80 kg N ha^{-1}
beginning of shooting (EC 31): N100: 15 kg N ha^{-1}, only in Warmse.
N200: both locations 30 kg N ha^{-1}
during shooting (EC 35): N100: Warmse 15 kg N ha^{-1}, Schoeningen 30 kg N ha^{-1}.
N200: both locations 30 kg N ha^{-1}
beginning ear emergence (EC 49): N100: both locations 30 kg N ha^{-1}
N200: both locations 60 kg N ha^{-1}

For protection against lodging, growth regulators (Chlormequat chloride + cholin-chloride = Cycocel) were applied during tillering depending on the N-treatment:
in N0 → 0.25 L ha^{-1}
in N100 → 0.5 L ha^{-1}
in N200 → 1.0 L ha^{-1}
so that lodging did not influence yield performance of cultivars.

A full application of fungicides on both locations kept the trials free of diseases. Fungicides were used as follows: against Pseudocercosporella herpotrichoides Sportak (Prochloraz) 1.5 L ha^{-1} at EC 32; against leaf diseases Corbel (Fenpropimorph) 1.0 L ha^{-1} at EC 35; against ear diseases Desmel + Dyrene (Propiconazol + Anilazin) 0.5 + 4 L ha^{-1} at EC 59 in both; an additional application of Bayfidan + Dyrene (Triadimenol + Anilazin) 1.0 + 4.0 L ha^{-1} at EC 75 in Warmse was necessary.

Investigated parameters

For grain yield two replications were harvested.

Grain samples per variety, treatment and location were analysed by Near Infrared Reflexion (NIR) for nitrogen content. Grain nitrogen yield has been calculated using the values for grain yield and nitrogen content in grain dry matter.

One replication was used for determination of dry matter and sequential nitrogen uptake in four vegetative stages during the growing period. For this purpose two samples of 0.312 sm each were harvested per cultivar, N-treatment and location: during tillering EC 27, at shooting EC 35, at ear emergence EC51 and at ripening EC 95. The results of these investigations will be presented in a later paper.

For characterization of the efficiency of mineral fertilization in additional consideration of the delivery and efficient use of the soil borne nitrogen, the Nitrogen Use Index (NUI) has been calculated: it is the relation of grain nitrogen yield of a cultivar in a certain nitrogen treatment to the sum of nitrogen found in the average of all cultivars in the zero treatment (N0) as grain nitrogen yield plus the total mineral nitrogen fertilizer:

$$\text{NUI} = \frac{\text{grain nitrogen yield in Nn}}{\text{mean grain nitrogen yield in N0} + \text{total amount of mineral nitrogen fertilizer}}$$

For statistical analysis of grain yield and grain nitrogen yield the computer program PLABSTAT of Utz (1986) has been used.

Results and discussion

Absolute values for grain yield in q/ha and for grain nitrogen yield in kg/ha are presented in Tables 3 and 4 and the summarized analysis of variance is in Table 2.

Table 2. Analysis of variance

Source	Grain yield		Grain nitrogen yield	
	F-value	LSD 5%	F-value	LSD 5%
Locations (L)	2.17 ns	23.9	2.22 ns	38.3
Nitrogen (N)	66.04**	10.3	157.35**	16.3
Cultivars (C)	10.49**	4.6	3.08**	7.7
LN	1.76 ns	14.5	3.30 ns	23.0
LC	0.90 ns	6.5	1.85+	10.8
NC	1.79*	5.3	4.73**	9.0
LNC	1.55	7.5	2.76**	12.7

Locations — nitrogen treatments

The reduction of nitrogen input resulted in the mean of all cultivars and all locations in a significant decrease of grain yield (Table 3, Fig. 1) at

Table 3. Absolute values for grain yield in q/ha at 86% DM under different N-conditions

Cultivars	Location	N-input			Mean
		N_0	N_{100}	N_{200}	
Apollo	Schoe	63.6	78.9	108.3	83.6
	Wa	50.1	71.9	95.2	72.4
	mean	56.9	75.4	101.7	78.0
Ares	Schoe	62.0	78.5	103.4	81.3
	Wa	49.3	79.9	102.3	77.1
	mean	55.6	79.2	102.8	79.2
Bernina	Schoe	60.2	79.9	101.7	80.6
	Wa	47.5	72.9	87.2	69.2
	mean	53.8	76.4	94.4	74.9
Brimstone	Schoe	66.2	85.5	105.8	85.8
	Wa	56.0	80.3	91.4	75.9
	Mean	61.1	82.9	98.6	80.8
Diplomat	Schoe	58.3	70.2	91.9	73.5
	Wa	45.9	69.7	80.9	65.5
	mean	52.1	69.9	86.4	69.5
Jubilar	Schoe	61.9	77.6	97.7	79.1
	Wa	47.6	76.3	92.1	72.0
	mean	54.7	76.9	94.9	75.5
Kraka	Schoe	62.6	79.9	108.9	83.8
	Wa	51.6	91.1	101.1	81.3
	mean	57.1	85.5	105.0	82.5
Kronjuwel	Schoe	53.2	79.2	103.5	78.6
	Wa	48.0	78.7	91.3	72.7
	mean	55.6	79.0	97.4	75.6
Milan	Schoe	65.2	81.8	109.6	85.5
	Wa	50.9	85.0	93.1	76.3
	mean	58.1	83.4	101.3	80.9
Obelisk	Schoe	60.7	84.4	109.5	84.9
	Wa	55.0	85.7	96.6	79.1
	mean	57.8	85.1	103.1	82.0
Rektor	Schoe	61.7	73.9	105.1	80.2
	Wa	48.9	77.8	94.9	73.8
	mean	55.3	75.8	100.0	77.0
Sorbas	Schoe	68.5	86.4	109.1	88.0
	Wa	52.4	82.2	97.1	77.2
	mean	60.4	84.3	103.1	82.6
Astron	Schoe	73.1	79.7	104.1	85.6
	Wa	45.7	78.1	93.7	72.5
	mean	59.4	78.9	98.9	79.0
Hybrid	Schoe	81.7	93.5	114.4	96.5
	Wa	60.8	95.0	106.4	87.4
	mean	71.3	94.2	110.4	92.0
Mean	Schoe	64.2	80.7	105.2	83.3
	Wa	50.7	80.3	94.5	75.2
	mean	57.4	80.5	99.9	79.3

Table 4. Absolute values for grain nitrogen yield in kg/ha DM under different N-conditions

Cultivars	Location	N-input			mean
		N_0	N_{100}	N_{200}	
Apollo	Schoe	94.7	124.9	202.0	140.5
	Wa	68.0	114.4	190.8	124.4
	mean	81.4	119.6	196.4	132.5
Ares	Schoe	91.2	127.6	184.9	134.5
	Wa	66.1	118.2	203.2	129.1
	mean	78.6	122.9	194.0	131.8
Bernina	Schoe	102.4	136.1	190.6	143.0
	Wa	71.1	113.5	179.2	121.2
	mean	86.7	124.8	184.9	132.1
Brimstone	Schoe	100.7	115.4	180.2	132.1
	Wa	76.6	116.0	176.1	122.9
	mean	88.7	115.7	178.1	127.5
Diplomat	Schoe	106.7	115.4	188.1	136.7
	Wa	70.6	112.0	169.0	117.2
	mean	88.6	113.7	178.5	127.0
Jubilar	Schoe	110.2	120.1	179.7	136.7
	Wa	69.1	119.4	176.6	121.7
	mean	89.7	119.7	178.1	129.2
Kraka	Schoe	90.9	122.9	190.1	134.6
	Wa	72.7	133.2	193.9	133.3
	mean	81.8	128.1	192.0	134.0
Kronjuwel	Schoe	80.5	132.8	195.9	136.4
	Wa	66.8	126.8	186.9	126.8
	mean	73.6	129.7	191.4	131.6
Milan	Schoe	107.1	131.5	182.8	140.5
	Wa	69.6	116.9	163.4	116.2
	mean	88.4	124.2	173.1	128.5
Obelisk	Schoe	83.5	127.0	201.6	137.3
	Wa	75.2	127.5	196.9	133.2
	mean	79.3	127.3	199.2	135.3
Rektor	Schoe	101.3	117.6	207.8	142.2
	Wa	71.8	118.4	188.5	126.2
	mean	86.6	118.0	198.2	134.2
Sorbas	Schoe	111.3	120.4	195.1	142.2
	Wa	73.5	128.7	187.9	130.0
	mean	92.4	124.6	191.5	135.1
Astron	Schoe	120.1	124.7	203.2	149.3
	Wa	69.1	129.0	182.1	126.7
	mean	94.6	126.8	192.6	138.0
Hybrid	Schoe	120.9	124.7	200.7	148.8
	Wa	77.4	144.6	197.7	139.9
	mean	99.1	134.6	199.2	144.3
Mean	Schoe	101.5	124.3	193.0	139.6
	Wa	71.3	122.7	185.1	126.4
	mean	86.4	123.5	189.1	133.0

the high absolute mean level of 100 q ha^{-1} (N200), the grain yield in N100 was 20% less; further, the 'zero' plots produced 57.4 q ha^{-1} (= 57% of N200) which is a very high yield potential for the locations. Although 'locations' and 'locations × N-input' are not significant (Table 2), the high level of Schoeningen with 64.2 q ha^{-1} in N0 confirms our experience with the high productivity of this location, based on a high mineralization ability of this loess loam soil and a high nitrogen-intensive crop rotation.

The grain yield of N0 in Warmse was 50.7 q ha^{-1} which is high for a poor sand with low organic matter; but the former crop was faba beans (*Vicia faba* L.) and the area received high rainfalls during 1987 (10.1986–9.1987: 729 mm) in combination with a rather cool and long growing period. The high mean values for grain nitrogen yield (86.4 kg N ha^{-1} in the plots without mineral nitrogen, Table 4, Fig. 2) indicated a very high level of soil borne-nitrogen at the locations, Schoeningen being significantly higher than Warmse. The increase of grain nitrogen yield up to 123 kg N ha^{-1} in N100 and 189 kg N ha^{-1} in N200 also revealed a high utilization of mineral nitrogen fertilizer in both N-treatments. The average Nitrogen Use Index indicated a better use of mineral nitrogen fertilizer for Warmse than for Schoeningen (Table 5).

Comparison of nitrogen content in grain from N0 plots (Table 5) between Warmse and Schoeningen confirmed a higher nitrogen mobilization activity of Schoeningen; no increase of nitrogen content in the grain was observed from N0 to N100, but a significant increase occurred from N100 to N200, similar for both locations. Variation for grain and plant nitrogen content in each N-treatment and each location occurred, differentiating among cultivars in each environment. The percentage of grain nitrogen content to total nitrogen content decreased in N200 for both locations (Table 5) and confirmed that the nitrogen translocation in the grain was higher in all cultivars under low nitrogen conditions (N0, N100).

Cultivars

Averaged over N-treatments and locations (Table 6), the relative grain yield ranged between 88% and 116%, confirming significant differences among cultivars; the old German cultivars Jubilar and Diplomat showed low grain yields. The high relative grain performance of the Hybrid might indicate future goals for hybrid breeding programs to increase wheat yield potentials.

Cultivars are generally judged by their grain yield performance and quality classification. But the ranking among cultivars changed with regard to the effective use of nitrogen (grain nitrogen yield): the range of relative grain nitrogen yield was between 95% and 109%, smaller, differences among cultivars were significant, but ranking of cultivars was different for nitrogen yield than for grain yield: the high performance cultivars Brimstone and Milan are at the bottom of the test cultivars for grain nitrogen yield, Rektor and Astron (high quality cultivars) had high grain-N-yield; the Hybrid, Sorbas, Obelisk and Ares had similar grain and grain nitrogen yield.

Cultivars × nitrogen input

Cultivars showed a significant interaction to nitrogen input for grain yield and grain nitrogen yield over locations (Tables 2–4).

Grain yield. With a decrease in nitrogen input, the grain yield of all cultivars decreased significantly, but most cultivars performed similarly for each N-treatment: the Hybrid and the cultivars Sorbas and Obelisk were at the top; cultivars Diplomat, Jubilar and Bernina showed poor performance over all nitrogen levels (Fig. 3–5).

Grain nitrogen yield. All cultivars have shown a significantly high reduction of grain nitrogen yield in the low N-treatments N100 and N0. The different reduction rates per cultivar and N-treatment, originating mainly from interactions cultivar × nitrogen input for grain nitrogen content, have caused a shifting of ranks for grain nitrogen yield performance in each N-input (Table 7).

Differences in N-input initiate larger differences among cultivars for grain nitrogen yield than for grain yield and a poor correlation exists between grain yield and grain nitrogen yield (Table 6). Some characteristic genotype-specific interactions for grain yield and grain nitrogen yield in different N-levels are demonstrated in Figs. 6 and 7. They indicate the necessity for the breeder to select cultivars that have high similar relative performances independently from nitrogen input for both

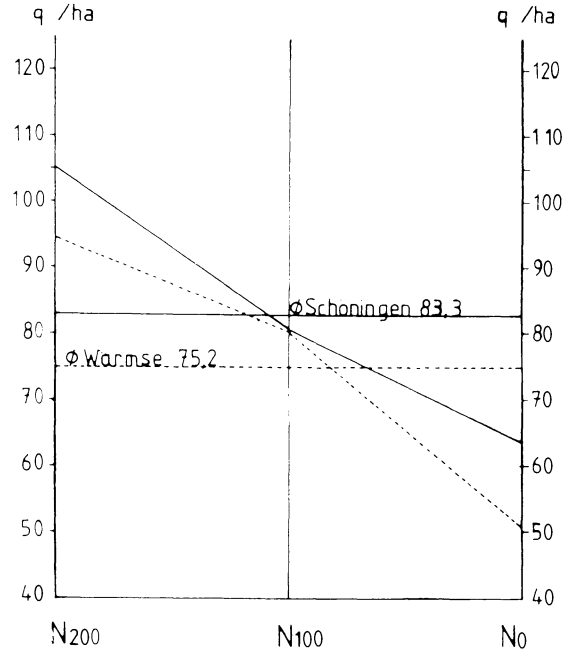

Fig. 1. Mean grain yield in q/ha at 86% DM under different N-conditions.

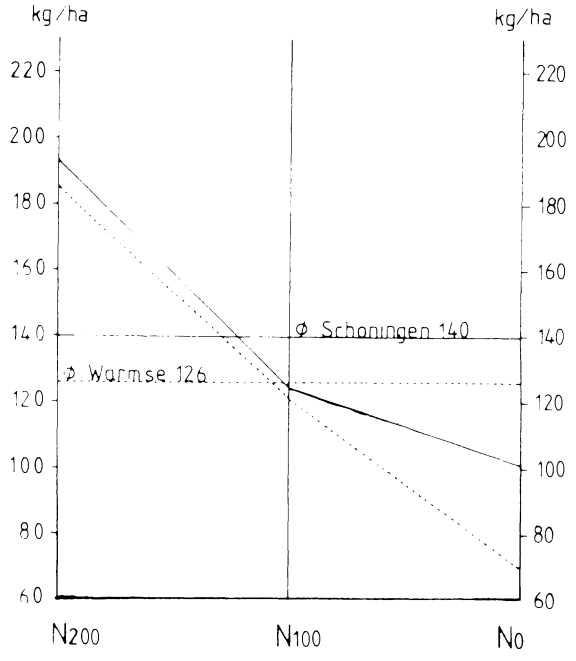

Fig. 2. Mean nitrogen yield in kg/ha DM under different N-conditions.

Table 5. Nitrogen Use Index and total nitrogen content i. DM grain and plant depending on location and treatment

Treatment	Location	Nitrogen Use Index		Grain nitrogen		Plant nitrogen		Grain: total nitrogen in %	
	Value	Wa	Schoe	Wa	Schoe	Wa	Schoe	Wa	Schoe
N_0	x̄			1.64	1.77	0.21	0.35	88.6	89.4
	range	—	—	1.48–1.76	1.71–2.07	0.10–0.31	0.29–0.42	—	—
N_{100}	x̄	0.72	0.62	1.78	1.80	0.20	0.35	89.9	83.7
	range	0.66–0.84	0.57–0.68	1.60–1.92	1.62–1.98	0.12–0.34	0.30–0.39	—	—
N_{200}	x̄	0.68	0.64	2.28	2.14	0.73	0.59	75.7	78.4
	range	0.60–0.75	0.60–0.69	2.04–2.43	1.94–2.38	0.57–0.96	0.48–0.74	—	—

Table 6. Relative performance of cultivars for grain yield and grain nitrogen yield. Means of locations and N-treatments

Cultivars	Quality group	Grain yield	Grain N-yield
Hybrid	B 4	116	109
Sorbas	B 4	104	104
Kraka	A 6	104	
Obelisk	B 4	103	
Brimstone	B 4	102	102
Milan	B 5	102	101
Astron	A 7	100	100
Ares	B 4	100	99
Apollo	C 2	98	
Rektor	A 9	97	97
Kronjuwel	B 4	95	96
Jubilar	B 4	95	95
Bernina	B 4	94	
Diplomat	A 7	88	
Mean = 100 %		79,3 q/ha	133 kg N/ha

parameters, grain and grain nitrogen yield (Sorbas, Fig. 7) or combine a good grain performance with a higher grain nitrogen performance at all nitrogen levels (Astron, Fig. 6). Nitrogen Use Indices (NUI) are calculated for the cultivars by location (Table 8). Significant differences among cultivars were found but no differences among N-levels existed. Some new cultivars (Brimstone, Milan) have low values similar to the old cultivars Jubilar and Diplomat.

A representative generalization of these results for cultivar specific characterization of effective use of nitrogen is not possible, since year effects may influence a different reaction. Results have to be proved in further trials.

A general conclusion for genetic improvement of nitrogen utilization can be drawn: Breeding for resistance properties and for resistance to lodging is an essential contribution to better nitrogen utilization and lower inputs. Previous breeding improvement through selection for grain performance has

Table 7. Relative performance of cultivars for grain nitrogen yield under different N-input. Mean of locations

N_{200}		N_{100}		N_0	
Hybrid	105	Hybrid	71	Hybrid	52
Obelisk	105	Kronjuwel	69	Astron	50
Rektor	105	Kraka	68	Sorbas	49
Apollo	104	Obelisk	67	Milan	47
Ares	103	Astron	67	Jubilar	47
Astron	102	Sorbas	66	Diplomat	47
Kraka	102	Milan	66	Brimstone	47
Sorbas	101	Bernina	66	Rektor	47
Kronjuwel	101	Ares	65	Bernina	46
Bernina	98	Apollo	63	Apollo	46
Brimstone	94	Diplomat	63	Kraka	43
Diplomat	94	Jubilar	63	Obelisk	42
Jubilar	94	Rektor	62	Ares	42
Milan	91	Brimstone	61	Kronjuwel	39
Mean: 189,1 kg/ha = 100%		Mean 65%		Mean 46%	

LSD 5%: 9,0 kg/ha = 5% rel.

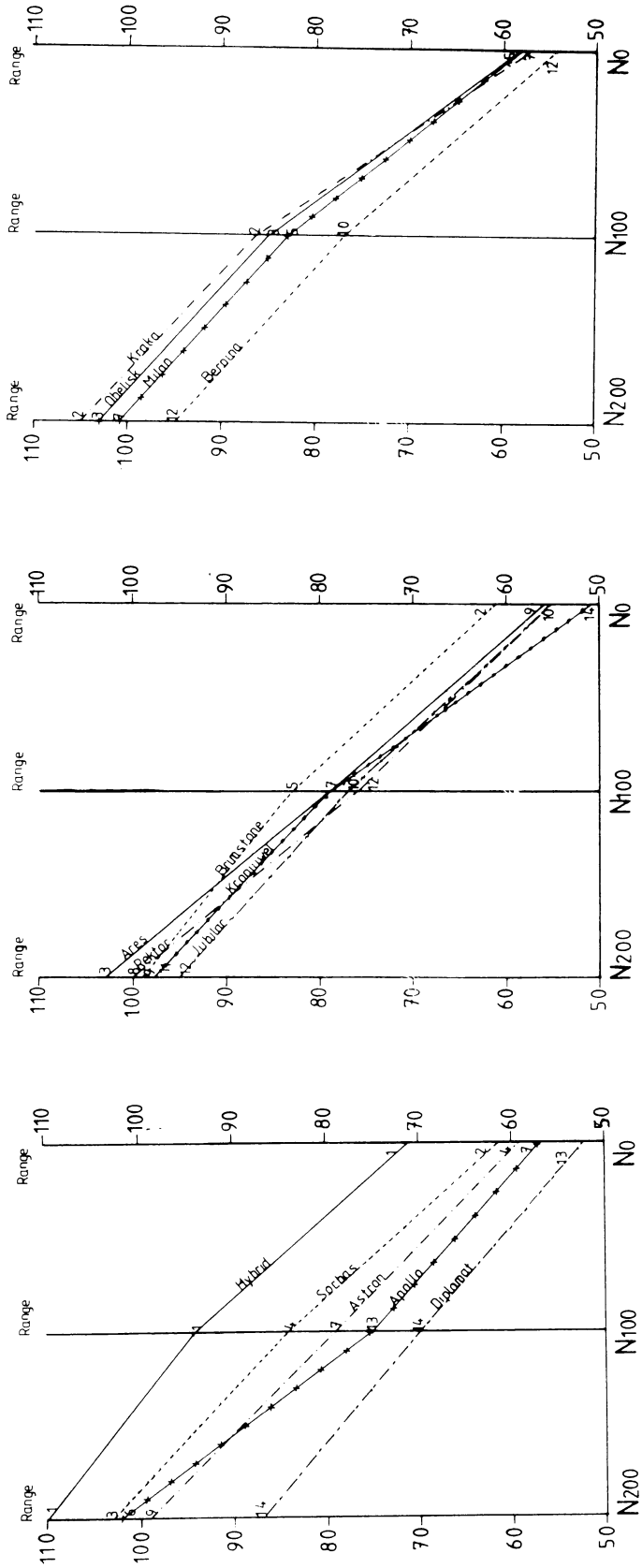

Fig. 3–5. Relative performance of cultivars for grain yield under different N-input. Means of locations: N_{200} = 99.9 q/ha = 100%.

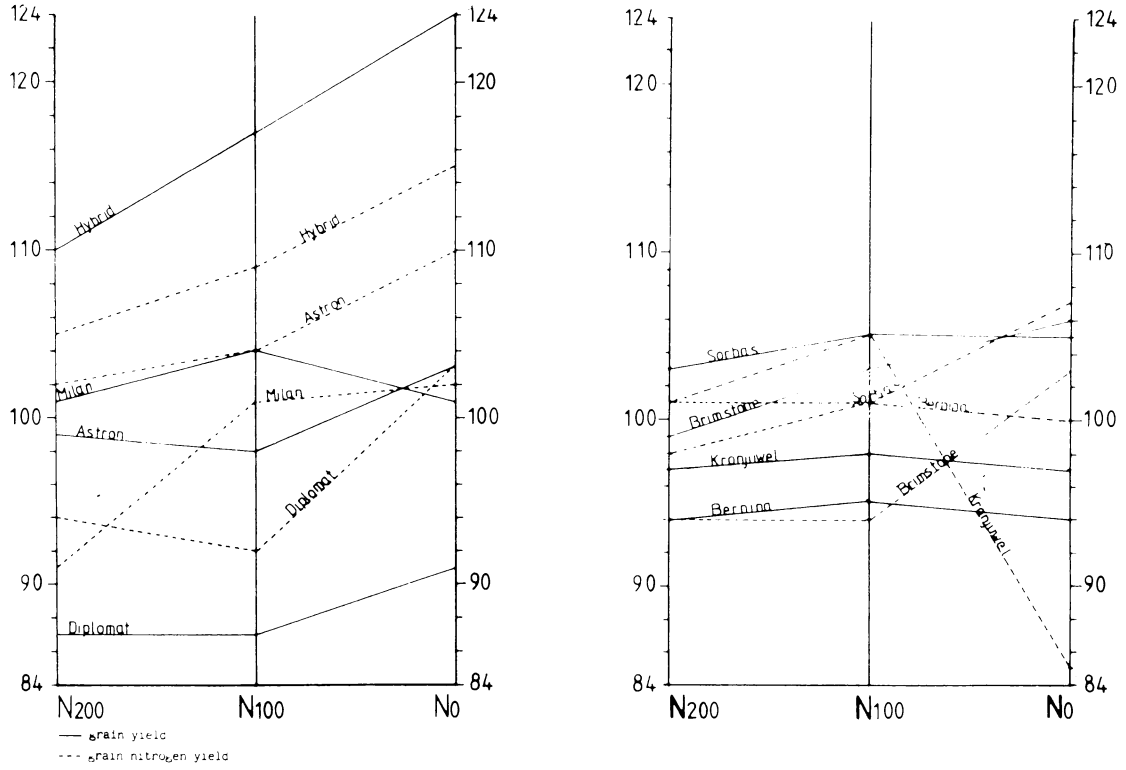

Fig. 6–7. Relative performance of cultivars for grain yield and grain nitrogen yield under different N-input resp. mean of each N-treatment = 100%.

Table 8. Nitrogen Use Indices (NUI) of cultivars in mean of locations

Cultivars	N-treatment		Mean
	N_{100}	N_{200}	
Hybrid	0.72	0.70	0.71
Obelisk	0.68	0.70	0.69
Kronjuwel	0.70	0.67	0.69
Astron	0.68	0.67	0.68
Kraka	0.69	0.67	0.68
Sorbas	0.67	0.67	0.67
Ares	0.66	0.68	0.67
Apollo	0.64	0.69	0.67
Rektor	0.63	0.69	0.66
Bernina	0.67	0.65	0.66
Milan	0.67	0.60	0.64
Jubilar	0.64	0.62	0.63
Brimstone	0.62	0.62	0.62
Diplomat	0.61	0.62	0.62
Mean	0.66	0.66	0.66

been very effective. However, breeders should consider whether they have to combine high grain performance with high grain nitrogen performance; since grain nitrogen yield has a low heritability breeders must maintain a broad-based testing system involving many differentiating low nitrogen environments.

The determination of Nitrogen Use Indices as an additional parameter might increase effectiveness of selection.

Acknowledgements

We thank Professor Röbbelen, Institute of Agronomy and Plant Breeding, University Göttingen, for cooperation and scientific advice.

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Suitability of varieties of winter wheat in low external input systems in West Germany*

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Key words: baking quality, disease resistance, ecological agriculture, low external input systems, vesicular arbuscular mycorrhiza, winter wheat varieties, yields

Abstract

Low external input systems are able to reduce significantly negative impacts of agriculture on the environment and economics (*e.g.* water pollution and overproduction). In West Germany they are represented by so called ecological agriculture. Yield, disease resistance, root growth, infection with vesicular arbuscular mycorrhiza, and baking quality of old and modern varieties of winter wheat were studied in different field trials from 1982 to 1987. Modern varieties (registered 1973–1983) gave higher grain yields and had better disease resistance and baking quality than older ones. Total root length was not reduced generally during the period of plant breeding with the extensive use of chemicals. There were little differences in the extent of mycorrhizal associations of four wheat varieties of different time of registration. Tall cultivars with high biomass production and good disease resistance properties were most suitable among modern varieties in ecological agriculture. Good baking qualities according to the official classifications were associated with a rather low protein content.

Introduction

Several problems concerning environmental damages and costs of overproduction, but also the quality of food and drinking water, may arise from 'modern' conventional agriculture with the extensive use of chemicals (Vogtmann, 1986). Low external input systems as represented in West Germany by ecological agriculture are managed according to the guidelines of the International Federation of Organic Agricultural Movements (IFOAM, 1985) and are able to reduce significantly many of these problems.

Genetic yield potential of winter wheat has increased markedly during recent decades. High input of fertilizers, herbicides, fungicides, insecticides, and other technological factors resulted in an enormous improvement of wheat yield in conventional agriculture. The genetic contribution to this yield increase was estimated to be approximately 40% (Schuster *et al.*, 1982; Silvey, 1978).

Growing conditions of winter wheat are different in a system without inputs of synthetic watersoluble N-fertilizers and biocides as compared to conventional agriculture. Thus characteristics of high yielding varieties of winter wheat with good baking quality might be different and even cultivars grown several decades ago might perform better in a low external input system than those bred in high input environments. Feyerherm *et al.* (1984) showed that environmental constrains greatly reduce the impact of genetic improvement.

Due to the dissimilarity of levels and flow of nutrients, rooting patterns and vesicular-arbuscular mycorrhizal (VAM) associations are likely to be of greater importance in ecological as

* Supported by the German Ministry of Nutrition, Agriculture, and Forestry, Bonn, FRG.

compared to conventional agriculture, because nutrients available to a crop depend primarily on the volume of soil occupied by roots and to a certain extend VAM hyphae. Close connections exist between growth above the surface and root growth of wheat (Chloupek, 1972; MacKey, 1973; Dambrodt and El Bassam, 1983). Therefore, wheat breeding including reduction of plant height, might have led to a decreased development of root systems.

Information on characteristics of winter wheat varieties suitable in low external input systems are very important for the development of an ecologically sound agricultural system because winter wheat is one of the economically and nutritionally most important crops. Studies were conducted with different groups of varieties of winter wheat on farms with low external inputs to gain basic information on the performance of wheat varieties in such systems.

Materials and methods

Material and experimental sites and design

Management practices were performed as usual at ecological farms. Ranges of plot size were 10–14 m^2. Drilling dates ranged from the beginning to end of October and drilling rates from 400 to 550 viable grains per m^2 according to local experiences. On the whole chemical and physical soil properties were suitable to wheat, except for the experimental site of VAM investigations. For more detailed information see Stöppler (1988).

Old and modern varieties of winter wheat. The comparisons of performance of old and modern varieties of winter wheat included 24 genotypes (Table 1), and was conducted at two farms in northern Hessia (FRG), using a randomized incomplete block design with four replicates per variety, except in 1984 (randomized complete block design with three replicates):

— at Oberhone from 1982 to 1983 (180 m above sea level, average temperature 9.2°C, average precipitation 554 mm $year^{-1}$, soil type Cambisol, sandy loam), a typical mixed family farm converted to ecological agriculture in 1975; preceding crops were two years of alfalfa-grass mixture in both years; neither the preceding crops nor the wheat crop received any inputs. No weed control was performed.

— At Neu-Eichenberg from 1983 to 1984 (240 m above sea level, average temperature 7.6°C, average precipitation 600 mm $year^{-1}$, soil type Cambisol, silty loam), the research farm of the Univ. of Kassel, Div. 'Methods of Alternative Agriculture', converted to ecological agriculture in 1981; preceding crops were field beans in 1982 and clover-grass mixture in 1983; neither the preceding crops nor the wheat crop received any inputs. No weed control was performed.

Investigations of rooting patterns. Investigations of rooting patterns, dry matter production, tillering, and yield formation were conducted using four winter wheat varieties: Landsorte (grown before 1900, very long straw), Carstens VI (released 1940, long straw), Vuka (released 1975, medium straw length), and Ural (released 1977, short straw). They

Table 1. Varieties of winter wheat and their year of release

Variety	Year of release		Variety	Year of release
Landsorte	before 1900[a]		Merlin	1956
Carstens II	1905[a]		Strubes Farino	1965
Carstens V	1921		Diplomat	1966
Svaslöfs Kronen	1926		Caribo	1968
Hauters II	1938		Kormoran	1973
Rimpaus Bastard II	1939		Saturn	1973
Heines IV	1940		Maris Huntsman	1975
Carstens VI	1940		Vuka	1975
Derenburger Silber	1941		Disponent	1975
Peragis	1943		Ural	1977
Breustedts Werla	1950		Okapi	1978
Pfeuffer Schernauer	1954		Milan	1983

[a] grown, no official release.

were grown in a randomized complete block design with six replicates from 1984 to 1985 at Oberhone and Neu-Eichenberg (description of sites see above). In these trials preceding crops were an oat-pea mixture in 1983 and field beans in 1984 at Oberhone, and clover-grass mixture in 1983 and potatoes in 1984 at Neu-Eichenberg. The oat-pea mixture received 12 and the potatoes 30 tons of FYM. No other inputs were given to the preceding crops or the wheat crop. Weed control was normally omitted, except in plots for root growth investigations, where it was done by hand. Thistles were removed generally.

VAM investigations. The experimental site of VAM investigations from 1985 to 1986 was located on a silty loam on clay with occasional water logging caused by insufficient draining (Cambisol) at Neu-Eichenberg. Lactate soluble P content was very low (17.5 ppm in 1985 and 8.7 ppm in 1986) and humus content high (3.7–4.0%) because the field was cropped with grass up to 1983. Crops prior to the wheat were potatoes in 1984 and winter rye in 1985. The field received no inputs from 1980 to 1986. Weed control was performed with a machine hoe. The four winter wheat varieties Landsorte, Carstens VI, Vuka, and Ural (see above) were grown in a randomized complete block design with four replicates in 1985 and six replicates in 1986.

Modern varieties of winter wheat. From 1985 to 1986 20 modern varieties of winter wheat (Table 2) were grown at 7 ecologically managed farm in West-Germany in 1985 and 8 in 1986 using a randomized complete block design with six replicates per variety. Some information on the experimental sites is given in Table 3. The wheat crop received inputs only at Rethem (liquid FYM 20 $m^3 ha^{-1}$), Contwig (preceding crop was a clover-grass mixture and used as mulch in 1985), Münsingen (FYM 15 t/ha), and Hergertswiesen (little FYM). Preceding crops others than potatoes were manured at Passade (FYM) and Rethem (liquid FYM); potatoes received FYM at every site. Weed control was performed only in Oberhone and Neu-Eichenberg, where it was done with a machine hoe.

Table 2. Varieties of winter wheat and classification of baking qualities according to the official description (volume of bread per unit of flour: low-medium = B4, very high = A9; Anonymous. 1985)

Variety	Quality category	Variety	Quality category
Monopol	A9	Oberst	A6
Rektor	A9	Vuka	A6
Severin	A9	Basalt	B5
Urban	A8	Granada	B5
General	A7	Heiduck	B5
Sperber	A7	Milan	B5
Disponent	A6	Nimbus	B5
Falke	A6	Ares	B4
Kanzler	A6	Kronjuwel	B4
Kraka	A6	Okapi	B4

Methods

Plots were harvested with a plot combine. Grain and straw yields wre adjusted to 86% dry matter content. Disease incidence was estimated according to official recommendations and growth stages as described by Zadoks *et al.* (1974). Elements of yield formation and plant height were investigated using samples of 1-m length of a drill row, each from three different sections per plot.

Root growth was examined in 1984 at Neu-Eichenberg and Oberhone at three sampling times and in 1985 at Oberhone at four sampling times using the profile wall method (Böhm, 1979) with four replicates. Biomass production patterns were investigated by harvesting 0.4 $m^2 plot^{-1}$ three times in 1984 and 0.625 $m^2 plot^{-1}$ four times in 1985. Final biomass production was calculated by adding grain and straw yield. Values were adjusted to 100% dry matter content.

Vesicular-arbuscular mycorrhiza was investigated from 1985 to 1986 at Neu-Eichenberg. Two samples were taken from each plot three times per year from May to July, consisting of 10–15 plants each with roots from 0–15 cm soil depth, and the adhering earth. The above ground parts of the plants and a sample of the soil were analyzed for P content, the remaining soil was washed from the roots on a sieve after a 24 h period of soaking. The two samples from one plot were united and the roots cut to obtain a representative sample, and cleared and stained as described by Phillips and Hayman (1970). The stained roots were placed on a slide and examined by estimating the percentage of the VAM-infected root length of total root length 30 times per slide at 40 × magnification.

Table 3. Some characteristics of the experimental sites, preceeding crops, inputs to the wheat crop, and yield level of the trial with 20 modern varieties of winter wheat in West-Germany

Site and year of investigation		Altitude m	Average temp. °C	Average precipit. mm/a	Soil type (FAO-system)	pH	Preceeding crops	Input to wheat crop (per ha)	Yield t/ha[2]
Passade	1986	25	8.4	750	Luvisol, sandy loam	6.6	red clover	mulch	5.62
Rothenhausen	1986	35	8.3	660	Luvisol, sandy loam	6.0	field beans	none	3.58
Rethem	1985	28	8.8	720	Cambisol, loamy sand	5.3	field beans	18 m^3liq. FYM	4.82
	1986					5.3	beets	20 m^3 liq. FYM	5.26
Neu-Eichenberg	1986	240	7.6	600	Luvisol, silty loam	6.1	potatoes	none	5.47
Oberhone	1985	180	8.7	554	Cambisol, sandy loam	5.7	field beans	none	5.50
	1986				Fluvisol,	7.1	potatoes	none	5.43
Düren	1985	150	10.1	593	Luvisol, sandy loam	6.2	field beans	none	5.68
	1986					6.2	winter rape	none	7.10
Contwig	1985	260	8.8	850	Cambisol, loam	7.2	clover-grass	mulch	6.23
	1986[1]					7.3	potatoes	None	3.52
Münsingen	1985	730	6.0	800	Rendzina, clayey loam	6.2	red clover	15 t TYM	4.64
Hergertswiesen	1985	530	7.4	850	Luvisol, silty loam	6.4	alfalfa	little FYM	4.64
	1986[1]					6.3	potatoes	little FYM	2.29
Geiselbullach	1985	500	7.5	800	Rendzina, human rich loamy sand	7.2	potatoes	none	2.46

[1]Partly deficient trials, not considered in every correlation computation;
[2]Mean of 20 varieties.

Baking quality was investigated at the Federal Research Institute of Cereal and potato Processing at Detmold, F.R.G.

Statistical analysis was conducted according to Sachs (1984).

Results and discussion

Impact of plant breeding on performance of winter wheat varieties in low external input systems in West Germany

The hypothesis among ecological farmers, that older varieties of Winter wheat, which had been successful in traditional farming systems some decades ago, would perform better in modern low external input systems than modern varieties, could not be proven. On the contrary, there was a significant tendency towards higher yields of modern varieties of winter wheat than older ones (Table 4). This is mainly due to the increased harvest indices. Probably, the correlation between age (date of registration) and yield of winter wheat varieties would have been more pronounced with average yields above 4.49 t ha^{-1}.

Although some diseases, *e.g.* mildew, are of much less importance in low external input systems compared to conventional farming, improved disease resistance may have led to increased yields in modern wheat varieties (Table 4). However, it has to be noted, that some of the modern varieties have shown a very high susceptibility to disease.

In low external input systems the extent of the root development is considered very important for

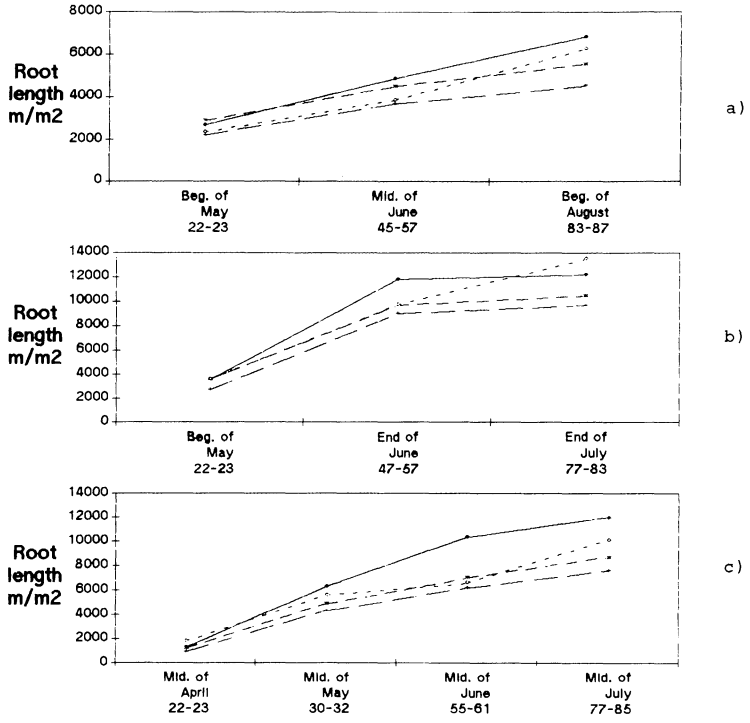

Fig. 1. Root length of four winter wheat varieties of different periods of release (m roots/m² soil; —— ● = Vuka; —— + = Ural; – – – ∗ = Carstens VI; – – – ○ = Landsorte). **a)** Neu-Eichenberg 1984; 0–40 cm soil depth; **b)** Oberhone 1984; 0–75 cm soil depth; **c)** Oberhone 1985; 0–120 cm soil depth.

plant nutrition. Obviously, during wheat breeding this development has undergone different modifications. For example Landsorte and Vuka showed considerable differences in rooting patterns (Fig. 1). In both years Landsorte showed only average growth of the root system until heading (1984) or shooting (1985), respectively, however a rather enhanced increase of total root length from heading until ripeness. This is in contrast to the dynamics of root growth in winter wheat described by Böhm (1978) and also to the root development of the other varieties tested, especially when compared with Vuka. It is easily understood, that this late root growth may lead to competition with grain development.

Table 4. Relations between years of release and some agronomic characteristics of 24 winter wheat varieties (Spearman's correlation of ranks; oldest variety rank 24)

Corr.: Year of release —	r (S, B)	
Grain yield	0.66***	1)
Susceptibility to powdery mildew	−0.51*	2)
Susceptibility to brown rust of wheat	−0.64**	3)

*, **, ***: sign. $\geqslant$ or $\leqslant 0$ at $P \leqslant 0.5, 0.01, 0.001$, respectively.
1) Oberhone, 1982–1983, and Neu-Eichenberg, 1983–1984;
2) Oberhone, 1983, and Neu-Eichenberg, 1984;
3) Oberhone and Neu-Eichenberg, 1983.

In 1985, the results of the four sampling dates show a very low root growth between shooting and heading of Landsorte and to a lesser extent also Carstens VI and Ural as compared to Vuka. The differences in root growth may be explained with the different tillering rates and development of tillers in all four wheat varieties (Belford *et al.*, 1987; Klepper *et al.*, 1984; Pinthus, 1969; Stöppler, 1988; Uprety *et al.*, 1983).

The data indicate that root length was not generally reduced by plant breeding. The rather tall modern variety Vuka showed high overall biomass production with an extensive root system, whereas the short variety Ural (released in 1977) was least developed in that respect at each sampling date. Carstens VI, released in 1940, showed an intermediate root development (Fig. 1). It is obvious, that the variety Vuka with an extensive root system established before the grain developing period is

well suited for a low external input system, which relies nearly exclusively on nutrient mineralisation for plant nutrition. This was clearly demonstrated by the higher yield potential of this variety.

The intensity of the development of vesicular-arbuscular mycorrhiza (VAM) in winter wheat varieties of different dates of release, is considered yield effective, if the soil contains rather low levels of lactate-soluble phosphorus (8.7–17.5 ppm; Fig. 2). The magnitude and dynamics of VAM infection in this study is in agreement with the results by Jakobsen and Nielsen (1983), who also consider it to be yield effective. Hayman (1970) found much lower infection rates in the roots of winter wheat before ripening and concluded that there was no effect on yield.

Because of the wide variability the data obtained do not allow a clear answer with regard to the influence of plant breeding on the possibility of a variety of winter wheat establishing an effective VAM association, although significant differences occurred during the dough stage in 1986. However, the possibility of varietal differences in VAM infection rates in winter wheat is confirmed (Azcon and Ocampo, 1981; Bertheau *et al.*, 1980; H W Dehne, Univ. Hannover, pers. comm., 1985).

In agreement with Hoeser *et al.* (1979) it can be concluded, that during the last decades not only an increase in grain yield but also an improvement in baking quality occurred due to progress in plant breeding (Fig. 3). Protein quality (Zeleny value) was better in most modern wheat varieties as compared to the older ones, whereas protein content was generally low (approximately 10%). Since in Germany wheat from low external input systems is almost exclusively used for the production of whole wheat flour and bread, the volume yield of such breed showed only slight differences. The excellent values of Landsorte are probably due to a very low grain yield.

Characteristics of suitability of varieties of winter wheat in low external input systems in West Germany

In conventional agriculture winter wheat is produced with high inputs of soluble nutrients and biocides to reach maximum yield through high plant density, a high number of grains per ear, and a high grain weight according to the genetic potential of the variety. In low external input systems, however, winter wheat develops the components of yield structure adapted to the flow of nutrients derived mainly from the organic matter of the soil, to the competition with weeds, and to the disease pressure.

Under these conditions it can be expected, that individual components of yield formation will be influenced to varying degrees. Especially plant density, but also the number of grains per ear, are established when the soil is still relatively cold and the mineralisation rate is low. The grain filling takes place normally under optimum conditions. However, at each time strong mutual influences can be assumed between the above ground dry matter

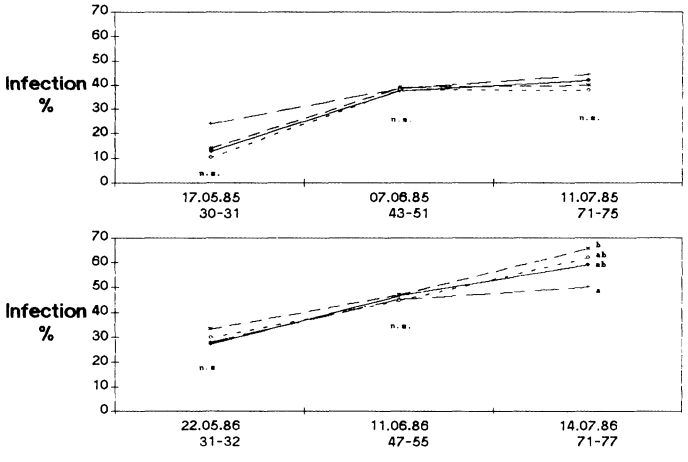

Fig. 2. Levels of VAM-infection of four varieties of winter wheat at different times or growth stages (percentage of infected root length; —— ● Vuka, —— + Ural, —— * Carstens VI, – – – ○ Landsorte; Neu-Eichenberg, 1985 and 1986).

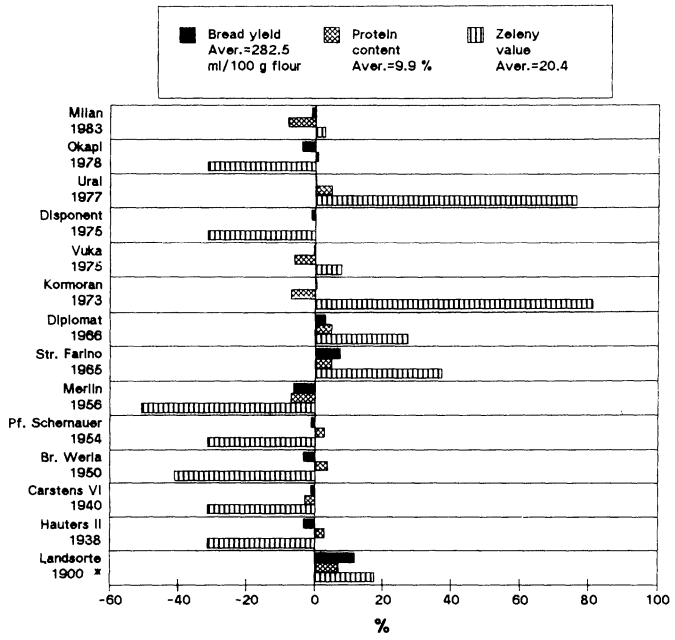

Fig. 3. Characteristics of baking quality of 14 winter wheat varieties of different periods of release (percentage differences to averages; Neu-Eichenberg 1984; year of release below variety name; *: grown before 1900; dates from Weismantel, 1985).

production and the intensity of root development.

In 20 modern varieties of winter wheat grain yield was significantly correlated with single grain weight, whereas this was not true for plant density and number of grains per ear (Table 5). However, with grain weight can be explained only 21% of the variability of grain yield (B = 0.21).

The positive correlation of grain yield with plant height is noteworthy. This may be explained by the improved competition of taller varieties with weeds, which is supported by the results of Wicks *et al.* (1986). They found highly significant correlations (r = 0.76) between grain yield and plant height and between suppression of weeds and plant height (r = 0.53) or grain yield (r = 0.68) with 20 varieties of winter wheat in the USA. Nevertheless, a very pronounced competition between winter wheat and weeds does not exist normally in ecological farming, because of the various agricultural practices used.

A very high correlation exists between grain yield and above surface dry matter production for the 20 modern and also for the 24 varieties of different times of release (Table 5). To eliminate any formal aspects of these correlations, the coefficients of correlation are also presented for grain and straw yield. Because of the tremendous increase in the proportion of grain weight on the total above ground biomass production, a differentiated evaluation of old and modern varieties of winter wheat is necessary. According to such an evaluation all correlations between grain and straw yield are significantly positive. There is no correlation between grain yield and harvest index in modern varieties.

The reason for these very clear positive correlations may be found in the correlation of both parameters — grain yield and above ground dry matter production — with the extent of the root system. This holds true for the above ground biomass production, although in some cases root weight or root surface and plant height have been used (Chloupek, 1972; Dambroth and El Bassam, 1983, Mac Key, 1973). Even with very careful interpretation of correlations of only four varieties, the correlation between total root length and above ground biomass production of winter wheat varieties of different periods of release, indicates the importance of this fact (Table 6). More research is needed for further clarification of these findings.

In this production system plant density of winter wheat generally reached only 300–450 ears m^{-2} and

Table 5. Relations between grain yield and characteristics of yield formation, harvest index, dry matter production, straw yield, and plant height of 20 modern varieties of winter wheat (7 sites in 1985 and 6 sites in 1986) and of 24 winter wheat varieties of different periods of release (Neu-Eichenberg, 1983–84)

Corr.: Grain yield —	Coefficient of correlation r
	20 modern varieties
Plant density	0.23
Grains/ear	0.04
Grain weight	0.46*
Plant height	0.60**
Harvest index[1]	−0.04
Dry matter production[1]	0.83***
Straw yield[1]	0.62**
24 varieties of different periods of release	
Dry matter prod. (all varieties)	0.70***
Dry matter prod. (12 varieties 1905–1956)	0.86***
Dry matter proc. (11 varieties 1965–1983)	0.93***
Straw yield (all varieties)	0.09
Straw yield (12 varieties 1905–1956)	0.51*
Straw yield (11 varieties 1965–1983)	0.78**

*, **, ***: sign. $\geqslant 0$ at $P \leqslant 0.5, 0.1, 0.01$, respectively.
[1] Oberhone, 1985, and Oberhone and Neu-Eichenberg, 1986.

good growth conditions with respect to nutrient flow are found normally from May to August. Therefore, the dynamics of root growth from shooting to ripening is assumed to be the key factor for achieving acceptable yield levels, and has to meet the demands of wheat plants for the development of a large number of grains per ear and especially during the grain filling period. Obviously, this is the case for varieties with high dry matter production and not for those genotypes with short straw and a low above ground biomass production.

At the Federal Research Institute of Cereal and Potato Processing at Detmold, F.R.G., studies with grain from the 20 modern varieties of winter wheat in 1985 (7 locations) and 1986 (8 locations) showed good milling quality and an overall satisfactory processing quality of flour type 550 as well as whole wheat flour. Although the total average of crude protein was only around 11% in both years and with average protein quality values (Zeleny value 26.3 in 1985 and 25.6 in 1986), the bread volume yield of flour type 550 was according to the official baking quality characteristics of the varieties used (Anonymous, 1985). In comparison with flour type 550, the use of whole wheat flour led to approximately half of the bread volume yield (Seibel and Stephan, 1985). Under these circumstances only the varieties with the highest quality level differed clearly from the others.

Table 6. Relations between total root length (TRL) and dry matter production (DMP) or four winter wheat varieties of different periods of release (growth stages at the times of investigations in parentheses)

Correlation	Coefficient of correlation (r)		
	Neu-Eichenberg 1984	Oberhone 1984	Oberhone 1985
TRL (77–83) — DMP (77–83)	–	0.83*	0.86*
TRL (83–87) — DMP (93)	0.99**	–	–

*, **: sign. $\geqslant 0$ at $P \leqslant 0.10, 0.01$, respectively.

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PLSO PN 190

Comparison between landraces and high yielding cultivars of winter wheat in extensive, integrated and intensive farming over several years

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Key words: breeding, landraces, modern cultivars, wheat

Abstract

Several German wheat cultivars are suitable for extensive and integrated cultivation methods of our area, but only a few of the 19 landraces-genotypes tested could be called "low-input-varieties".

The landraces are worse in *all* their characteristics (yield, resistance, a.s.o.) than the new varieties, but their gen-reservoir in single characteristics, such as extremely early bloom and maturity, resistance to mildew, high protein content, a.s.o. is excellent.

Introduction

Wheat grain surpluses in the EC and environmental pollution by high levels of nitrate in ground water should be reduced by selective measures, such as using less mineral fertilizer.

Are modern varieties suited for extensive cultivation? To what extent can the genetic potential of existing extensive landraces be used as "low-input" genetic material in today's cultivation of first-class quality wheat?

Materials and methods

Site

The experimental farm of the Nürtingen college is at Tachenhausen and the soil is little humus earth, clay, loam. The site is 330 m above sea level with an average annual temperature of 8,0 °C and an average annual rainfall of 750 mm.

Varieties

Leading A- and B-winter wheat varieties of German plant breeders, landraces from Baden-Württemberg, USSR, Turkey, mostly collected by the author.

Installation of experimental plot

Several experiments were carried out with the following three degrees of intensity: extensive/integrated/intensive.

Four repetitions were used with a row width of 14 cm using 400 germinable grains per m^2. Yield factors were analyzed together with determination of protein content. Infestation with diseases and resistance to lodging, time of blooming and ripeness were evaluated and the results of experiments are shown in the following tables.

Results and discussion

In field tests over several years, 23 important German cultivars of winter wheat were compared with 19 other genotypes cultivated in three areas. The listed varieties are significantly superior on yield to all landraces in the various degrees of intensity. They are similarly responsive to the increasing intensity of treatment (Tables 1 and 2). Some varieties proved to be especially well suited to

Table 1. Comparison of grain yield between modern cultivars and land races of winter wheat from field studies of the college Nütringen (Tachenhausen, 1985-1987)

Modern varieties			Various landraces		
Extensiv	Integrated	Intensiv	Extensiv	Integrated	Intensiv
100[a]	121	125	71	93	95

([a] 100 = 66, 1 dt ha^{-1})

Table 2. Variations

Variations of fertilization and treatment		
Extensiv	Integrated	Intensiv
Optimum base fertilizing of potassium phosphate 1 × weed control with herbicides 1 × measures to combat diseases of ears (*Erysiphe graminis*, *Septoria nodorum* a.o.) 1 × nitrogenous fertilizers: 30 kg N ha^{-1}		
	+ 20 kg N ha^{-1} + 40 kg N ha^{-1} (late fertilizing)	+ 60 kg N ha^{-1} + 40 kg N ha^{-1} (late fertilizing)
	+ 1 × regulator of growth + 1 × measures to combat stem break	+ 2 × regulator of growth + 1 × measures to combat stembreak + Treatment of leaves and second treatment of ears + 1 × pest control

Table 3. Comparison of qualities important in agriculture

	Modern cultivars (23)			Land races (19)		
Suitable for	Extensiv	Integrated	Intensiv	Extensiv	Integrated	Intensiv
Very high yield	8 + 1	8 + 2	8 + 4	1	2	(1)
Backing quality	12	14	16	3	4	4
High yield and backing quality	1 (3)	2 (5)	4 (7)	1	2	(1)
Very good resistance to lodging	6	–	–	2	–	–
Toleranz to-mildew	8	–	–	3	–	–
Very early bloom	0	–	–	4	–	–

extensive treatment by the factor "number of grain/ears".

Eight leading cultivars are superior in their yields in all variations, while only two of the landraces could hold their own to some degree (Table 3). Of 12 tested A-varieties we obtained the expected high amount of protein and sedimentation. But some landraces are better suited for baking in "normal years" than the best A-varieties. Only one landrace and few modern cultivars gave a high yield and are at the same time well-suited for baking.

Six listed varieties and two landraces are attested

as having good resistance to lodging and tolerance of mildew in the extensive variation and the extremely early blooming and ripeness of some landraces was particularly striking.

Acknowledgements

I would like to thank Mr H Gärtner, Mr M Wüstholz and Mrs A Ziegler for their help with the field experiments and GTZ Eschborn for financial support.

N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 489–495.
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PLSO PN 191

Breeding wheat (*Triticum aestivum*) for aluminium toxicity tolerance at CIMMYT

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Key words: aluminium, breeding, hexaploid, toxicity

Abstract

CIMMYT launched a breeding program in 1974 primarily to combine Al toxicity tolerance of Brazilian wheats with high yield potential and wide adaptability of Mexican semidwarfs. This experiment has been a success as numerous advanced lines and cultivars have been produced with at least a 25% increase in yield potential combined with increased tolerance to Al toxicity in acid soils. Laboratory screening methodology combined with shuttling of alternate segregating populations between locations in Mexico and Brazil were utilized as the breeding methodology. This paper outlines historical perspective, genetical variability in wheat varieties, screening methodology, shuttle breeding, and future prospects and development in Al toxicity research at CIMMYT.

Introduction

Crop development is influenced by the interaction of many factors, including such things as genotype, soil moisture, temperature, and mineral stress. Mineral stress in plants can be caused by either nutritional deficiencies or toxicities. Deficiencies of one or more essential plant nutrients are widespread in developing countries, resulting in low productivity in vast areas of 'worn out' soils. There are also large areas of the world, an estimated 2,960,800,000 hectares (Dudal, 1976), representing 22.46 percent of the world land area, that are adversely affected by mineral toxicities and/or deficiencies.

Among these problem soils are the highly leached acidic Oxisols and Ultisols, which are characterized by toxic levels of soluble aluminum and manganese. These soils cover approximately 1 billion hectares of the tropical and subtropical areas of Brazil, Southeast China, Southeast Asia, and Central Africa (Van Wambeke, 1976). Currently, these areas are either undeveloped for agriculture, or where cultivated, are of very low productivity. To meet a rapidly growing demand for food during the next four decades, these problem soils must be developed and their productivity improved. This can be done by a combination of varietal improvement, corrective chemcial fertilization, and improved management practices.

Aluminum and manganese toxicities are among the most important factors limiting the productivity of crop plants in many acid soils of the world (Da Silva, 1976; Foy, 1973; Foy, 1974; Kamprath and Foy, 1971; McLean, 1976; Olmos *et al.*, 1976). Aluminum toxicity is particularly severe below pH 5.0, but has been reported in soils with pH values as high as 5.5 (Adams and Lund, 1966; Hester, 1935). The current approach to correcting aluminum toxicity recommends changing the soil pH by liming, which is not always economically feasible, particularly in strongly acidic subsoils.

Fortunately a range of tolerance to aluminum toxicity has been found to be genetically controlled in a considerable number of species and varieties of crops (Camargo *et al.*, 1980; Kerridge *et al.*, 1976; Moore *et al.*, 1976). Aluminum toxicity severely

inhibits root growth (Foy, 1974a) by preventing cell division (Clarkson, 1965; Henning, 1975) and elongation in the root apical meristem. The drastically restricted root system that results makes the plant vulnerable to moisture stress and unable to utilize low levels of available essential plant nutrients.

Brazilian wheat breeders pioneered the development of selecting wheat varieties for tolerance to aluminum toxicity. This work was initiated in 1925 (Beckman, 1976) and over five decades resulted in developing a large number of tolerant varieties. Unfortunately, without exception, these traditional varieties possessed poor agronomic type and had low genetic grain yield potential. On the other hand, the CIMMYT wheat varieties possessed broad adaptation, high genetic grain yield potential and a broad spectrum of disease resistance but, without exception, were sensitive to aluminum toxicity and hence poorly adapted to acid soils. Fourteen years ago, CIMMYT, in collaboration with Brazilian wheat breeders, undertook a cooperative breeding program to combine the tolerance to aluminum toxicity of the Brazilian varieties with the high genetic yield potential and the broad adaptation of the CIMMYT varieties. Currently, there are many advanced generation wheat lines developed by this cooperative program that are in the final stages of testing. These lines combine tolerance to aluminum toxicity, good agronomic characteristics, a broader spectrum of disease resistance, and high yield potential. This paper summarizes the CIMMYT–Brazil cooperative breeding program, the screening procedures employed, and the results so far obtained.

Materials and methods

Germplasm

Two varieties of durum wheat (*Triticum durum* Desf.), 3 varieties of rye (*Secale cereale*) 2 varieties of triticale (× *Triticosecale* Wittmack) and 5 varieties of bread wheat were used in the preliminary study of the aluminum toxic reaction in different species of cereals (Table 1). In addition 7 hexaploid wheat varieties from Brazil with known field resistance to aluminum and one Mexican variety with known sensitivity to aluminum were employed to study correlation of response between field reaction and laboratory condition (Table 2). Three hundred and fifty six advanced lines of bread wheat with supposed tolerance to aluminum were employed to study genetic variability to aluminum tolerance (Table 3). These advanced lines were selected alternately in Brazil and CIMMYT. And finally 12 families of crosses with variable number of advanced lines were studied for yield potential and disease resistance (Table 4).

Table 1. Reaction of crop plant cultivars to three levels of soluble aluminum

Crop	Cultivar	Aluminum (ppm)		
		38	51	62
Bread wheat	Maringa	T	T	S
	CNT 8	S	S	S
	Alondra	S	S	S
	Pavon 76	T	S	S
	Jupateco 73	S	S	S
Durum wheat	Yavaros 79	S	S	S
	Mexicali 75	S	S	S
Rye	Snoopy	T	T	T
	Prolific	T	T	T
	Blanco	T	T	T
Triticale	Cananea 79	T	T	T
	Caborca 79	T	T	T

T = tolerant; S = sensitive.

Table 2. Field and laboratory reactions to aluminum toxicity of certain cultivars

Cultivars	Field reaction in Brazilian acid soil	Aluminum reaction 46 ppm
BR 12-4/BH1146*6/ALD F28292-A-799Y-103F-102Y-108F	T	T
JUP73*6/TP F28468-C-101F-103Y-103F	T	T
PF83144	T	T/MT
BR 23	MT/MS	T/MT
CEP 11	MT	T/MT/MS
CEP 14	MS/MT	T/MT
CNT 8	MS/MT	T/MT
JUPATECO 73 (Mexican)	S	S

T = tolerant; MT = medium tolerant; MS = medium sensitive; S = sensitive.

Table 3. Distribution of 356 advanced lines of bread wheat derived from Brazilian × Mexican crosses when tested at 34, 40 and 46 ppm of aluminum concentration

Classification	Aluminum concentration (ppm)		
	34	40	46
Tolerant	61	61	61
Medium tolerant	117	117	117
Sensitive	178	178	178
Total	356	356	356

Laboratory methodology for screening wheat seedlings to aluminum

The CIMMYT wheat breeding program uses a laboratory procedure in which seedlings are exposed to a low-pH nutrient solution containing high levels of aluminum. The main effects of aluminum toxicity in the nutrient solution is clearly detected because of reduced root growth and development of sensitive seedlings. Several methods were tested at CIMMYT laboratory before a modified hematoxylin method was adapted for screening purposes. The reagents and procedures are described below.

Reagents. Seven different solutions are prepared for this laboratory procedure. These are:

1. *Preparation of concentrated nutrient solution*: Dissolve in about 900 mL distilled water, 22.20 g $CaCl_2$ anhydrous, 32.86 g KNO_3, 25.41 g $MgCl_2 6H_2O$, 0.66 g $(NH_4)_2SO_4$, and 1.60 g NH_4NO_3. This is diluted to 1 liter and stored at 7°C.

2. *0.1 M aluminum solution*: Dissolve 24.14 g of $AlCl_3 6H_2O$ in 900 mL of distilled water. Dilute to 1 liter and store at 7°C.

Table 4. Advanced bread wheat lines showing tolerance to 46 ppm of aluminum concentration, combined with high yield potential, good plant type, and dwarfing genes

Cross	No. of selections	Disease resistance			Yield % Seri 82[b]
		PRT	PGT	ST	
DOVE'S'[a]/BUC'S' CM58808	7	R	R	S	100
PF70354/BOW'S'[a] CM67910	1	MR	R	R	95
CEP7780/BOW'S'[a] CM74254	3	R	R	MR	90
IAS 58/IAS 55//ALD'S'[a]/3/MRNG/4/ALD'S' /IAS 58.103A//ALD'S'/5/BUC'S' CM74362	16	R	R	MR	95
PF70354/MUS[a]//VEE#3[a] CM76603	3	R	R	MR	95
SERI[a]/PF70100 CM78418	9	R	R	MS	90
BUC'S'/BJY'S'//LIRA[a] CM78428	2	R	R	S	95
ESMERALDA/LIRA[a] CM78428	5	R	R	S	95
PF72640/PF7326//PF7065/ALD'S'[a]/3/KLT[a] CM81780	7	R	R	MR	90
IAS 58/4/KAL/BB//CJ/3/ALD'S'[a]/5/ BOW'S'[a] CM81812	51	R	R	MR	90
IAS 58/4/KAL/BB//CJ/3/ALD'S'[a]/5//CNR CM81818	36	R	R	MR	90
KVZ[a]//KAL/BB/3/CEP7596/4/CEP8064 B29553	7	R	R	MR	90

[a] Carrier of 1B/1R translocation.

[b] Yield potential of 8 tons/ha measured in Mexico.

R = resistant; MR = moderate resistant; MS = moderate susceptible; S = susceptible; PRT = *Puccinia recondita tritici*, PGT = *Puccinia graminis tritici*; ST = *Septoria tritici*.

3. *0.25 M HCl solution*: 20.83 mL of concentrated HCl diluted to 1 liter. Store at 7°C.

4. *0.2% hematoxylin solution*: Dissolve 2 g of hematoxylin anhydrous in 900 mL distilled water, add 0.2 g of NaO_3 and dilute to 1 liter.

5. *Nutrient solution*: Place 760 mL of distilled water in a plastic container, add 240 mL of the concentrated nutrient solution as prepared above. Add 11 liters of distilled water. Mix and adjust the pH to 4.0 using the 0.25 *M* HCl solution. The resulting concentration is: 4 m*M* $CaCl_2$, 6.5 m*M* KNO_3, 2.5 m*M* $MgCl_26H_2O$, 0.1 m*M* $(NH_4)_2SO_4$, and 0.4 m*M* of NH_4NO_3.

6. *Nutritive solution containing aluminum*: The 1.7 m*M* aluminum solution equal to 46 ppm of aluminum is prepared by mixing 760 mL distilled water, 240 mL of concentrated nutrient solution, 204 mL of the 0.1 *M* aluminum solution and then diluting to 12 liters with distilled water. Adjust the pH to 4.0.

7. *10% sodium hyopochlorite solution.*

Wheat seeds are sterilized with the 10% sodium hypochlorite solution for 30 seconds and rinsed thoroughly with water. The soaked seeds are then placed on wet filter paper in Petri dishes and kept at 7°C for approximately 65 hours.

The Petri dishes are removed from the refrigerator and the seeds left to germinate at room temperature (18–20°C) for approximately 24 hours. The sprouted seeds are placed on a polyethylene net that has been fixed in lucite frames. Styrofoam blocks are attached to the frames with rubberbands and floated on the surface of the nutrient solution in containers with constant aeration. These are placed in a water bath maintained at 25°C and exposed to 8 hours of incandescent light, per day. Four planting trays with 60 squares and 4 germinating seeds per square are placed in each container with 12 liters of nutrient solution. Two check varieties (one tolerant and one sensitive) are included in each tray.

After the seedlings have grown for 32 hours in the nutrient solution, they are transferred to a nutrient solution with 46 ppm of aluminum, which has a pH of 4.0 and maintained at 25°C. This is continuously aerated for 17 hours.

After 17 hours, the seedlings are removed from the nutrient solution containing aluminum, the roots thoroughly washed for 2–3 minutes in running tap water and stained with the 0.2% hematoxylin solution for 15 minutes. The excess dye is washed off with distilled water.

The seedlings are returned to the containers with distilled water or nutrient solution at 25°C and continuously aerated for approximately 24 hours. The seedlings are evaluated on a 1–3 scale where 1 is sensitive, 2 is medium tolerant and 3 is tolerant. The roots of type 3 seedlings continue to grow in nutrient solution while type 1 seedlings do not reinitiate growth. The type 2 seedlings have intermediate response. The roots of sensitive seedlings do not grow because the meristems have been irreversibly damaged.

Field methodology: Cooperative shuttle breeding program between CIMMYT (Mexico) and Brazilian institutions

Most traditional Brazilian wheat varieties tend to grow tall and lodge, usually tiller poorly and produce spikes with only two or three grains per spikelet. These defects, largely account for their low genetic yield potential. They are, however, in general tolerant to aluminum toxicity in Brazilian acid soils.

The CIMMYT wheat breeding program has attempted, during the last 14 years, to breed high-yielding, semi-dwarf, broadly adapted varieties with high levels of tolerance to soluble aluminum.

Crosses are made between aluminum tolerant Brazilian germplasm and broadly adapted, high-yielding, semi-dwarf CIMMYT wheats. Selections are made in segregating generations at two locations in Mexico: 1) the Mexican government's CIANO Research Center, Ciudad Obregon, Sonora, located at 28°N latitude and 40 meters above sea level, and 2) the CIMMYT Research Center at Toluca, State of Mexico, at 19°N latitude and an elevation of 2,560 meters. These locations are shown in Figure 1.

The wide adaptation and broad spectrum of disease resistance of the CIMMYT germplasm is attributable to the shuttling of genetic materials in alternate segregating generations between the two aforementioned Mexican locations, which are very diverse in climatic and ecological conditions. In Ciudad Obregon, the plantings are made in November when the diurnal photoperiod is becoming shorter, whereas the plantings in Toluca are

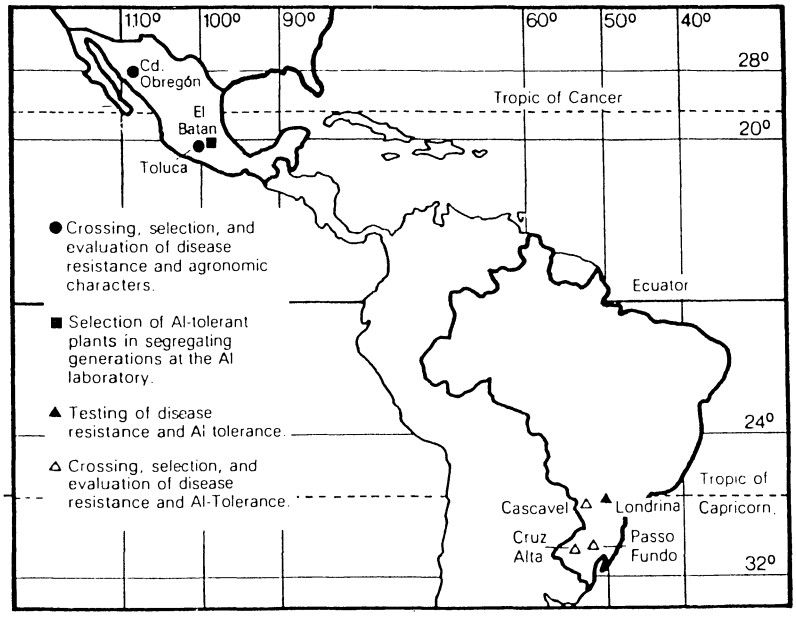

Fig. 1.. Mexican and Brazilian locations involved in shuttle breeding.

made in May when the diurnal photoperiod is becoming longer. Consequently, the shuttling of the progenies of alternate generations between these two locations helped identify photoperiod insensitive wheats with wide adaptability, high degree of yield stability and high grain yield performance.

The results from the laboratory screening tests combined with those of the cooperative shuttle breeding effort between CIMMYT and Brazil have been very positive. Promising segregating materials identified in the laboratory are selected and grown under different acid soil conditions in Brazil, and at the two non-acid sites at Toluca and Ciudad Obregon, Mexico.

Results and discussion

Correlation of response between field and laboratory screening

To execute proper screening in the laboratory, it is very essential that there is a high degree of association between the genotypic reaction to aluminum in the laboratory and in the field. To test this association, eight varieties of known field reactions were tested in the laboratory. Seven of these varieties were from Brazil and ranged in their aluminum reactions in the field from tolerant, medium tolerant or medium sensitive. The Mexican variety, Jupateco 73, was used as sensitive check. All seven Brazilian varieties were found to be tolerant, medium tolerant or medium sensitive (Table 2). The mixed reactions of Brazilian varieties are indicative of the heterogeneity in relation to the response to 46 ppm of aluminum in the laboratory. However, the data showed high association between field responses and laboratory reactions and hence the value of laboratory screening in the breeding of aluminum toxicity tolerant wheats.

Genetic variability within CIMMYT's hexaploid wheat for aluminum toxicity tolerance

Shuttle breeding between Mexico and Brazil and aluminum screening in the laboratory have made it possible to generate a series of advanced bread wheat lines which have acceptable tolerance to aluminum toxicity combined with high yield potential, dwarfing character, and resistance to 3 rusts and *Septoria tritici*.

Three hundred and fifty-six lines derived from CIMMYT × Brazilian crosses were tested in 3

aluminum concentrations of 34, 40, and 46 ppm to determine the spectrum of genetic variability. Sixty-one lines were tolerant, 117 lines were medium tolerant, and 178 lines were sensitive at all 3 concentrations (Table 3). The lines sensitive at 46 ppm were neither tolerant nor medium tolerant at 40 and 34 ppm. This suggested a narrow genetic base of the 61 tolerant and the 117 medium tolerant lines. Aluminum tolerance is stable, not vulnerable to break down as is common with biotic stresses, although the further introduction of diverse genetic aluminum tolerant sources in CIMMYT crossing program would be desirable. The genetic variability within the tolerant lines and between the tolerant and medium tolerant lines is not known. Future studies should focus on genetic analysis to characterize the varieties.

Yield potential of advanced lines with aluminum tolerance

Table 4 lists 12 selected crosses representing various advanced lines. Their rust and *Septoria tritici* reactions were measured in Mexico and their yields expressed as a percentage of Seri 82, a high yielding Mexican commercial variety, measured in Cd. Obregon, Mexico. As stated earlier, the yield of original Brazilian Al-tolerant wheats were very low (approximately 3–4 t ha^{-1} in Mexico). The yield of the best derivatives has essentially doubled, now 90–95% of the best check variety, Seri 82, in the Yaqui Valley of Mexico. All of these advanced lines have the required rust resistance and some possess acceptable tolerance to *Septoria tritici* as well.

Incorporation of 1B/1R translocation with aluminum tolerance

All the lines listed in Table 4 carry the 1B/1R translocation. This is characterized by the union of a small piece of the 1R chromosome of rye with small arm of 1B chromosome of wheat. Experimental data have indicated that the 1B/1R-translocated wheats have shown intermediate tolerance to aluminum toxicity. Since this tolerance is different than that derived from Brazilian sources, it is assumed that a combination of aluminum tolerance genes from Brazilian varieties and 1B/1R translocation could provide positive additive effects and hence overall better aluminum tolerance. More research will be required to measure the interaction and possible additive effects of the 1B/1R translocation and the traditional aluminum tolerance gene of the Brazilian varieties.

Acknowledgements

We sincerely thank Ing Jaime Lopéz-Cessati for carrying out the aluminum toxicity tests in laboratory and Dr Norman E Borlaug for his advise and critical review of the manuscript.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 497–499.
PLSO PN 192
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Buckwheat — a low input plant

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Key words: allocation, buckwheat, determinant, germplasm, ideotype, nitrogen, phosphorus, potassium, screening, sodium

Abstract

Buckwheat can be grown on soils poor in nutrients, sandy soils and on stony fields. It is a good utilizer of insoluble phosphorus. When it is grown on soil too rich in nitrogen, it can easily become lodged. Some buckwheat genotypes are capable of rapid plant development. All these characterize buckwheat as a low input plant.

Buckwheat germplasm resources were collected and evaluated for development of genotypes with lodging resistance and higher productivity. Genes for the determinant growth habit were found, expressing pleiotropic effects: cessation of further growth of the apex at some stage of development, altered shape and size of terminal inflorescence, earlier and more uniform development of kernels, larger kernels, shorter plants, plants more resistant to lodging and with development less sensitive to photoperiod. The influence of the gene for determinant growth habit on low input characteristics of buckwheat is discussed.

Introduction

Buckwheat (family Polygonaceae) is grown and used in a similar way to cereals. Though it does not belong to Poaceae, it is often grouped together with cereals in agronomy and commerce. Common buckwheat or sweet buckwheat (*Fagopyrum esculentum* Moench) is a crop of some importance in Nepal, India, Pakistan, Afganistan, Iran, China, Japan, Korea, USSR, Poland, Hungary, Yugoslavia, Denmark, France, Canada, USA and Brazil. Some growing of buckwheat has also been reported from CSSR, Austria, Switzerland, Italy, Great Britain, Finland and Australia.

Tartary buckwheat or bitter buckwheat (*Fagopyrum tataricum* Gaertn.) is grown to a lesser extent as a crop (or mixed with common buckwheat) in Nepal and in Yugoslavia for food, and sometimes also in Poland as a source of rutin. But it exists as a weed amongst common buckwheat in many countries.

Buckwheat is a typical low input plant. Many buckwheat varieties have a very short vegetation period. Some domestic buckwheat populations in Yugoslavia need only about 60 days from sowing to harvest. It is an undemanding crop, grown on sandy soils poor in nitrogen and on stony fields but higher doses of nitrogen cause lodging. Buckwheat is a good utiliser of soil phosphorus (Andersen and Thomsen, 1978) but modest fertilising with phosphorus may sometimes improve yields. Buckwheat roots have good K/Na selectivity (Eggers and Jeschke, 1983).

Common buckwheat needs a relatively high temperature for germination (about 10 °C), but at optimal temperatures (about 20 °C) and moisture it may emerge in 2 or 3 days after sowing (Kreft, 1986). It has relatively low optimal temperatures (Kajfež-Bogataj and Gaberščik, 1986) and is thus chilling resistant, but it is not frost resistant, being damaged at temperatures of about −2 °C (Gaberščik *et al.*, 1986).

Buckwheat has optimal photosynthetic intensity at temperatures of 15 °—25 °C (Kajfež-Bogataj and Gaberščik, 1986). Buckwheat is not sensitive to diseases and pests and there is normally no need for application of insecticides, fungicides or herbicides. In Yugoslavia, most damage to buckwheat is caused by roe deer, and sometimes by birds.

Buckwheat has low yields, mostly in the range of

0.5 to 2 tons per hectare and yields have not changed much in this century. In this time, wheat yields have increased five to ten fold. Common buckwheat is a cross pollinated self-incompatible plant and thus is not suitable for improvement by conventional plant breeding methods. It has a poor response to high levels of fertilisers, has an indeterminant growth habit and is not lodging resistant. The ripening of kernels is not simultaneous and often the first ripe kernels fall off before the last kernels are ripe. Buckwheat plants usually have many more flowers than they are capable of bearing as filled kernels. Overproduction of flowers is a considerable waste of organic matter, water and minerals from the point of view of the allocation of photosynthates, although some part of these are probably removed from flowers before they desiccate.

In short: buckwheat today is much more similar to a weed or even to a wild plant than to a modern high-yielding crop.

For improving buckwheat yields it is necessary:

— to form an ideotype of high yielding buckwheat,
— to use modern techniques for overcoming self-incompatibility, crossing, and other barriers and problems,
— to screen germplasm for desirable genes.

It would be of particular interest to improve buckwheat yields, but not to change the low-input characters of this crop.

Materials and methods

Buckwheat germplasm resources were collected, conserved and screened. Included in the screening were samples from Austria (1 sample), Brazil (1), China (2), India (3), Italy (1), Japan (8), Nepal (2), Poland (5), USSR (11) and Yugoslavia (16).

Scores were allocated for:

— length of vegetation period (in days from sowing to first dark coloured kernels),
— growth habit (determinant/indeterminant),
— lodging (estimated),
— side branching,
— height of plants,
— seed setting (as number of seeds per inflorescence and mass of seeds per plant).

Experiments were performed between 1982 and 1987 on the experimental field of the Biotechnical Faculty, Ljubljana, Yugoslavia, on heavy soil, low in mineral nutrients and organic matter, under moderate drought conditions during flowering time. Each sample was tested in at least two years, in two replicates and compared to buckwheat cv. Siva. Interesting samples were also included after screening in the crossing programs.

Results

Large variation was found among the screened material in time from sowing to ripening. The shortest (about 60 days) was for some samples of black hulled buckwheat from Yugoslavia, even a few days less than the control cv. Siva. But in comparison to Siva, they had a much lower yield and were less resistant to drought and poor soil conditions. In Yugoslav climatic conditions, one of the samples from India was unable to set seed in autumn; 110 days after sowing, the plants were still intensively flowering.

We were unable, by the method of screening as described, to distinguish samples which are clearly superior in drought and poor soil conditions. However, we obtained information about material suitable for further testing by more sensitive test methods. The determinant forms were at least not inferior in drought and poor soil conditions.

In screening buckwheat germplasm, several genes controlling buckwheat growth habit were found. Lodging resistance could be improved by dwarfing genes and by genes for the determinant growth habit. Dwarf plants have shorter internodes and slower development. Types with a determinant growth habit are very promising. Determinant plants were found in three samples from Yugoslavia and in a sample from Italy.

The studied recessive gene for the determinant growth habit from a sample of Yugoslav origin has pleiotropic effects. Cessation of further growth of the apex and of development of new leaves and inflorescences; altered shape of the terminal inflorescence; earlier and more uniform ripening of kernels, less shedding of kernels; higher number of kernels per inflorescence; shorter plants more resistant to lodging; development of plants, flowering and ripening time are less sensitive to photoperiod; the harvest is relatively early, while the leaves are still green. There is also a characteristic lack of

apical dominance. After the cessation of further growth of the apex of the main stem, more intensive side branching occurs.

Discussion

The determinant buckwheats studied exhibit characteristics which may be the basis for an ideotype of high yielding buckwheat.

More intensive branching may be controlled by higher density; the higher optimal density in determinant buckwheat may be an important factor in achieving higher yields. A higher density in buckwheat may even increase the existing good competitive ability of buckwheat in comparison to most weeds.

Determinant buckwheat is lodging resistant, which enables higher yields through a higher rate of fertilisers. However, this may cause buckwheat to lose one of the important characteristics of low-input plants. In determinant buckwheat, leaves may be green and photosynthetic effective until the end of the grain filling period. This is good from the aspect of production of photosynthates, but may indicate that some nitrogen in the leaves could not be translocated in time to the filling kernels. This nitrogen (and other minerals) which remains in the leaves at harvest time is used less effectively, unless the green leaves are also used for some purpose (forage, buckwheat tea, rutin production etc.).

Since intensive buckwheat breeding is to be expected in the near future, it is important also to study and consider the eventual genetic variability in the efficiency of utilisation of mineral nutrients, as far as possible to retain in high yielding buckwheat the characteristics of a low-input plant.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 501–521.
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PLSO PN193

Phosphorus: A limiting nutrient in bean (*Phaseolus vulgaris* L.) production in Latin America and field screening for efficiency and response

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Key words: beans, phosphorus, Al-toxicity

Abstract

Bean (*Phaseolus vulgaris* L.) is a staple food in Latin America and is frequently grown by small farmers on acid infertile soils. Average yields are very low ($\pm 500\ kg\ ha^{-1}$) when compared to those in temperate zones ($1500\ kg\ ha^{-1}$). On acid, infertile soils, the main limiting factor for bean production is low available phosphorus. These soils have a high P-fixing capacity, so additional P from fertilizers is soon unavailable. Furthermore, the residual effect of P fertilizer is practically nil. P fertilizer is expensive and many farmers cannot afford to apply it. Other soil constraints (*e.g.* pH, Al toxicity, and low organic matter) impede P use efficiency and response to applied P. Also, fertilizer management influences the response of beans to P application.

Differential yield response of beans in the field and in the greenhouse to P fertilization has been observed, but poor correlation is found as to dry matter production, leaf area index, P content in the tissue, and P uptake by the plant. Screening techniques for controlled environments do not give satisfactory results when tested in the field. The difficulties of these methods proposed the simple field screening method but the efficiency gain is small. Some other potential mechanisms for screening are discussed.

Introduction

Beans in Latin America are mostly grown on acid soils (*e.g.* oxisols and ultisols) and, to a lesser extent, on entisols, alfisols, and inceptisols. These acid soils are characterized as being low in P, high in P fixing capacity, high in Al, and sometimes also high in both Al and Mn (Sanchez and Cochrane, 1980). The major constraints reported for growing bean on these soils are low pH (Mueller *et al.*, 1968), low P (Fassbender, 1967; Jacob and Uexkuell, 1963; Malavolta, 1972; Thung *et al.*, 1984), and N deficiency (Diaz-Romeu *et al.*, 1970).

Malavolta (1972) reviewed more than 232 fertilization trials in Brazil. He found that 73% of the trials gave positive responses to either P or N fertilizers (44% to P and 29% to N). The IBGE statistics yearbook (1986) indicates the evident necessity of fertilization for bean production in Brazil which is responsible for 35% of the world production. During the period of 1970 to 1986 total bean production fluctuated between 1.6 to 3.1 million tons while the total production area increased form 3.5 to 6.3 million ha. According to Miyasaka *et al.* (1966), the response of beans to P fertilization is high compared to other nutrients under Brazilian conditions. CIAT (1978) showed a response variation from 1.3 to 8.7 (kg yield/kg P fertilizer) depending on growth habit, seed color, and season.

On the other hand, beans are not the most productive legumes grown under soil stress conditions. CIAT (1981) compared the 5 most commonly grownt legumes, which belong to 3 different genera, *Vigna*, *Glycine*, and *Phaseolus*,

under low soil P and high Al and Mn conditions. Several contrasting lines from the genera *Glycine* and *Phaseolus* were included. *Glycine max*. "ICA L-129", *Phaseolus vulgaris* cv. "Carioca" and "G 4000" represented the cultivars tolerant to acid soil conditions and *Glycine max*. "ICA Tunia" and *Phaseolus vulgaris* "Puebla 152", and "BAT 47" represented the non-tolerant lines. Results showed that P stress is more detrimental to yield that Al and Mn stress for all species (Fig. 1). *Vigna unguiculata* and *Phaseolus angularis* beans were the only two

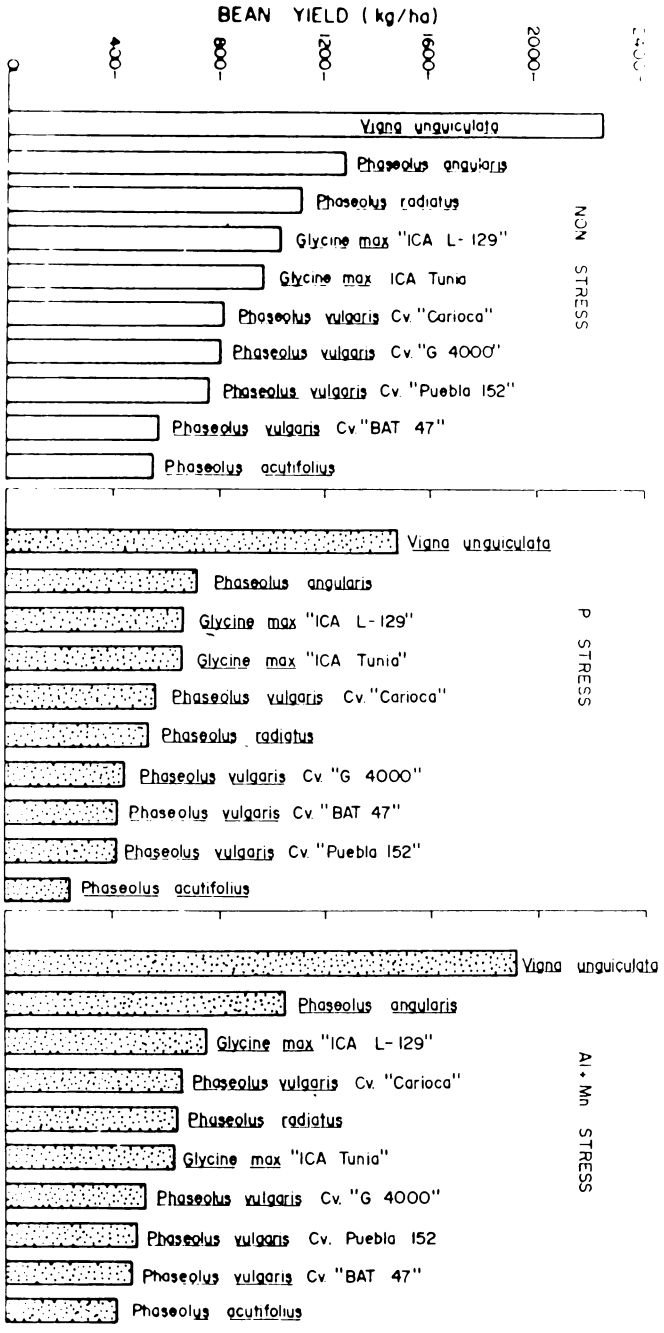

Fig. 1. Yield (kg ha^{-1}, 14% Hum) of various grain legumes grown under three different soil stress conditions (CIAT 1981).

species that performed well under both P and Al + Mn stress and non-stress conditions. These results also suggest that beans are not too poorly adapted to adverse soil conditions and that tolerant cultivars may be widely adapted to different environments.

Although it is quite clear that P is the principal limiting factor, breeding work emphasizing this constraint is minimal. The problem is that breeding for soil constraints, including P, is very difficult, since reliable selection criteria are still lacking. This paper deals with P requirements, some possible methods of screening, and prospects for breeding work.

P requirements for growth and production

Studies of P absorption by the bean plant have been conducted by many authors under field as well as greenhouse conditions (Batista, 1974; Cobra Netto, 1967; Feitosa *et al.*, 1980; Gallo and Miyasaka, 1961; Haag *et al.*, 1967; Malavolta, 1985). From these studies it can be concluded that the amount of P absorbed by the bean plant is very small (10–20 kg P ha^{-1}) in comparison to the amounts of N and K (200 kg N or K/ha) (Fig. 2 and Table 1). The actual loss of P from the field in the harvested grain is small, about

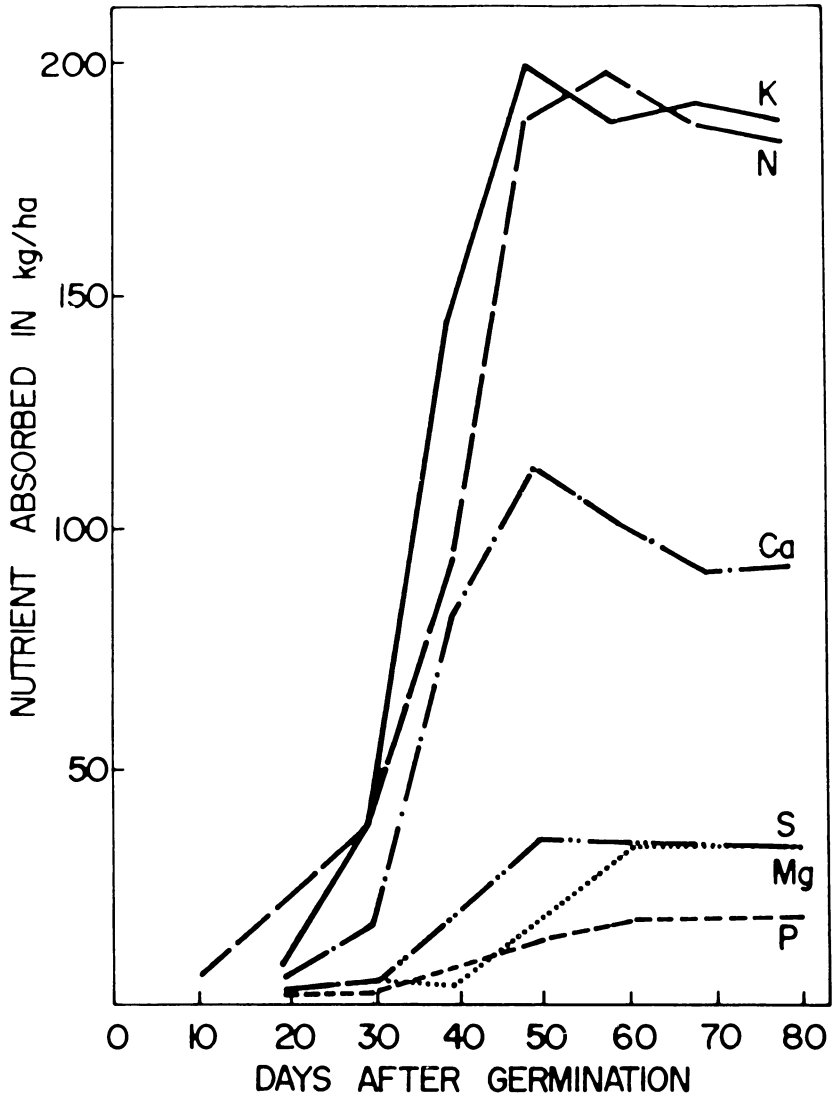

Fig. 2. Nutrients absorbed by the bean plant during the growing season. (Haag *et al.* 1967).

Table 1. Total nutrient absorption by the bean plant (*Phaseolus vulgaris* L.)

Author	Density	Nutrients (kg ha^{-1}) N	P	K	S	Ca	Mg
Field experiments							
Cobra Netto 1976	250,000	102 (1.00)	9 (0.09)	93 (0.91)	26 (0.26)	54 (0.53)	18 (0.18)
Gallo and Miyasaka 1961	–	84 (1.00)	7 (0.08)	68 (0.81)	6 (0.07)	34 (0.41)	11 (0.13)
Feitosa *et al.* 1980	200,000	81 (1.00)	7 (0.09)	62 (0.76)	– –	57 (0.70)	10 (0.12)
Greenhouse experiment							
Haag *et al.* 1967	250,000	201 (1.00)	18 (0.09)	201 (1.00)	36 (0.18)	116 (0.58)	36 (0.18)
Relation among nutrients		1.00	0.09	0.83	0.17	0.55	0.14

Table 2. Nutrient removed from the field by bean at a scale of 1000 kg ha^{-1}

Author	Nutrients (kg ha^{-1}) N	P	K	S	Ca	Mg
Cobra Netto 1967	34.2 (1.00)	3.4 (0.10)	20.3 (0.59)	8.8 (0.26)	4.1 (0.12)	4.1 (0.12)
Haag *et al.* 1967	37.2 (1.00)	3.6 (0.10)	22.0 (0.59)	9.5 (0.26)	4.4 (0.12)	4.4 (0.12)
Feitosa *et al.* 1980	22.0 (1.00)	3.9 (0.18)	13.2 (0.60)	– –	3.0 (0.14)	2.0 (0.09)
Mascarenhas *et al.* 1969	35.4 (1.00)	3.8 (0.11)	18.7 (0.52)	– –	1.3 (0.04)	1.7 (0.05)
Mean of nutrients carried out	32.2	3.7	18.6	9.2	3.2	3.1
Relation among nutrients	1.00	0.11	0.58	0.29	0.10	0.10

4 kg P/1000 kg seed (Table 2). Additional losses due to soil erosion are unknown.

The rate of P absorption is slow during the early stages of plant growth, begins to accelerate 25 days after emergence, and culminates during flowering (0.27–0.63, 2.17–4.39, and 4.39–6.53 kg P ha^{-1} during each of these preiods, respectively), as shown in Table 3 (adapted from Corbra-Netto, 1967).

Although beans require only small amounts of available P in comparison to other elements, this requirement must be fulfilled until maturity whereas requirements for N, K, Ca, and S decrease after flowering (Fig. 2).

P fertilization

P fertilization for beans is widely recommended to improve yield (Fontes *et al.*, 1965; Guzman, 1980; Miranda and Lobato, 1978; Miyasaka *et al.*, 1964; and Miyasaka *et al.*, 1966). Beans responded more to P application than to other elements (Malavolta, 1972). However, due to poor agronomic practices and economic reasons, especially for the small farmer, P fertilization is not attractive. In many cases, fertilization does not give positive responses because of irregular or late rain, or total lack of precipitation, inadequate doses of P applied, high P-

fixing capacity of the soil, or high levels of Al in the soil which is not amended by liming.

The method of application also plays an important role in high P-fixing soils, such as oxisols. Banding of phosphate fertilizer is recommended to increase efficiency (Saman, 1963). Lathwell (1979) reported that beans responded well to banded P and an efficiency increase of 2 1/2-fold was obtained on a red-yellow soil of the central highlands of Brazil. A 1400 kg ha^{-1} bean yield was obtained from 175 kg broadcast P ha^{-1}, the equivalent of only 70 kg banded P ha^{-1}. Differences between varieties (Rico 23, Carioca, Rosinha, and Roxinho) were almost nil, suggesting that the benefits of banding P fertilizer do not vary with the bean genotype planted. The same results were obtained in Puerto Rico, where banded applications of 79 kg P ha^{-1} gave the same results as broadcasting at a rate of 489 kg P ha^{-1} (del Valle *et al.*, 1977, cited by Lathwell, 1979). Deep placement (25 cm) of water soluble forms of P fertilizer also improved bean yield, and no yield depression occurred at higher P dosages as was observed with superficial applications (Thung *et al.*, 1982) (Fig. 3). However, applying fertilizer a long time before planting is not recommended because of the high P-fixing capacity of the acid soils. CIAT (1984) showed that in soils with either low or high P content the best application time for water-soluble phosphate fertilizer is at the time of planting (Fig. 5). The later the time of application relative to the planting date, the lower the yield. P application at flowering produced yields similar to unfertilized controls (Figures 4 and 5). This indicates that late P application does not produce readily available P at the time that coincides with that of the high P demand by the plant, which begins at flower initiation (CIAT, 1985). On the other hand, phosphate fertilizer needs ample time before it becomes available to the plant.

P fixation by acid soils, which are deficient in available P in their native state, is a series problem, as discussed extensively by Sanchez and Uehara (1976). Once the initial deficiency is corrected, the commonly recommended dosage

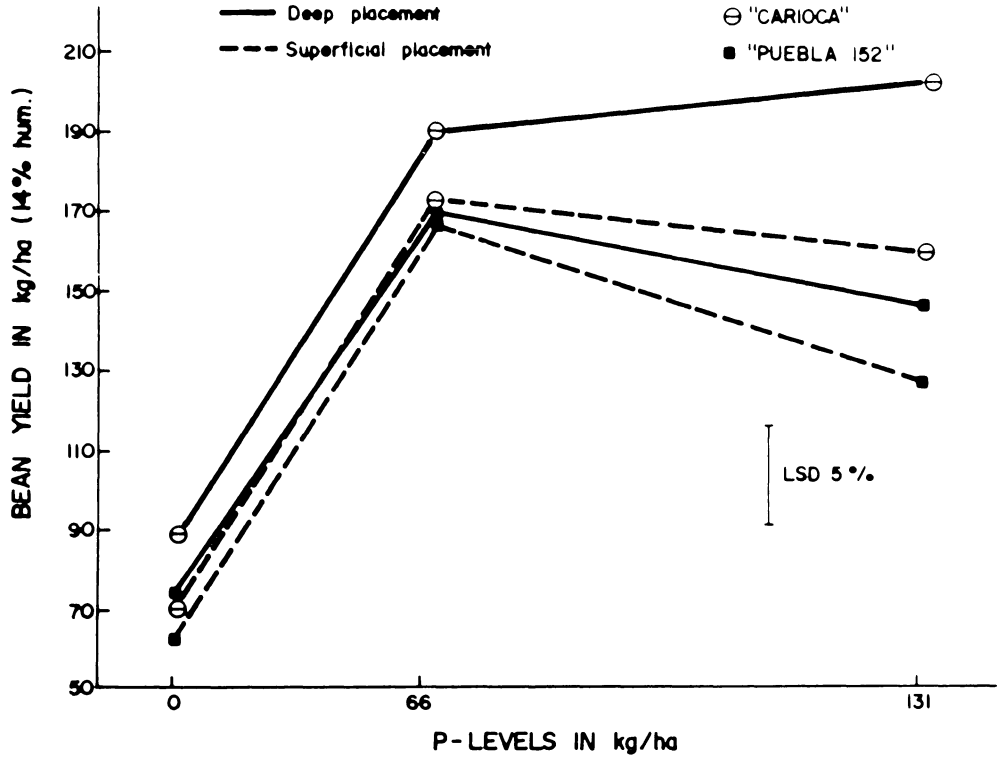

Fig. 3. Yield of two bean varieties affected by different application depths and three levels of phosphorus. (Thung *et al.* 1982).

Table 3. Absorption rate of phosphous (kg ha^{-1}) by bean plants during the growth cycle with 250,000 plants x ha^{-1} (adapted from Cobra Netto 1967)

Days after emergence	Total	Leaf	Stem	Flower	Pods	Roots
–						
0–15	0.27	0.19	0.06	–	–	0.02
15–25	0.63	0.47	0.10	–	–	0.06
25–34	2.17	1.78	0.30	–	–	0.09
34–46	4.39	3.26	0.64	0.40	–	0.09
46–56	6.53	4.60	0.58	0.15	1.05	0.15
56–66	2.86	0.65	0.33	–	1.84	0.04
66–77	3.97	0.11	0.18	–	3.66	0.02

for beans grown on P-deficiency oxisols is around 44 kg P ha^{-1}. Only about 20% of the total P (*viz.* from the soil and the applied fertilizer) is actually absorbed by the plants. Tinker (1981) reported that only 10% of fertilizer P is utilized by plants in temperate zones. Kick and Minhas (1972), Mandal and Kahn (1977), and Gill and Meelu (1983) also mentioned that the P use efficiency of applied P fertilizer varied between 10–30%. Studies of this type in the tropics are still rare.

P fertilization of beans on acid soils always increases yield when N is not a limiting factor. N fertilization improves the response of P fertilization and increases yield (Miyasaka *et al.*, 1967a). Figure 6 shows the range of varietal differences (12 lines) under different P levels (triple superphosphate) with limited (20 kg ha^{-1}) and sufficient (100 kg ha^{-1}) N (urea). The effect of P fertilization was greater than that of N (CIAT, 1978 and 1979).

On the other hand, there was no response to N and P application when errative rainfall occurred during 2 years of experimentation (Tarazona and Zanstra, 1974). The ratio of the P and N dosage generally recommended for fertilization is around 10:1, approximately the reverse ratio of which the bean plant absorbes (Table 1). When the amount of lime applied is inadequate excesive N fertilization may reduce yield on acid soils as a result of the acidifying effect of the applied N (urea) (CIAT, 1977). The acidity equivalent for urea fertilizer is approximately 75. This means that in order to keep the soil pH at the same level as before the N fertilization 75 kg of additional $CaCO_3$ equivalent is required for each 100 kg of urea fertilzier (*e.g.* acidity equivalent for ammonium sulphate and ammonium nitrate is 110 and 60, respectively).

The P source also influences bean yield. As a short growth period crop, water soluble P is

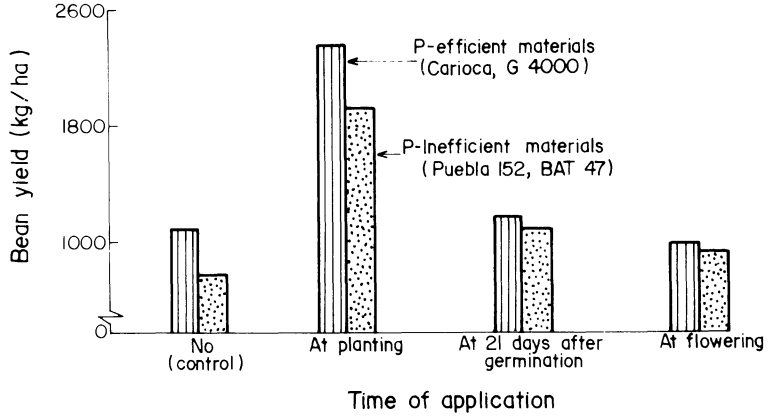

Fig. 4. Yields of efficient and inefficient bean varieties as affected by time of application of P fertilizer (applied in bands) on soil with low P content (4.2 ppm P Bray II). (CIAT 1981).

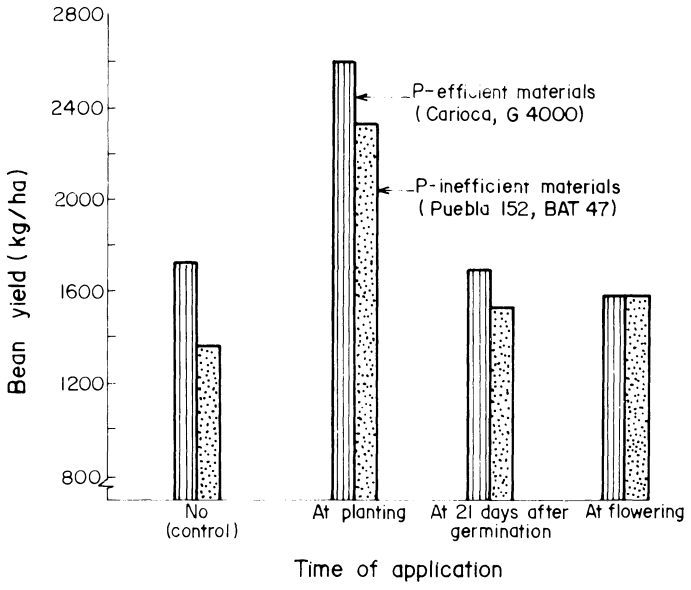

Fig. 5. Yields of efficient and inefficient varieties as affected by time of application of P fertilizer (applied in bands) on soil with high P content (25.6 ppm P Bray II). (CIAT 1981).

preferable (Atanasiu *et al.*, 1978). Water soluble P is the best option. Some alternative sources, especially when applied on acid soils, give satisfactory results. CIAT (1977) compared 5 sources of rock phosphate to water soluble phosphate during 4 consecutive years. Bean yield increases corresponded to the citrate solubility of the P source. Measuring the residual effect through 4 consecutive plantings showed that the highly soluble P forms, such as triple superphosphate or Gafsa rock, became less effective. Hence, the initial differences in effectiveness of the P source decreased over time and yields decreased. Broadcast or banded applications of a mixture of equal parts of water soluble phosphate and rock phosphate were proposed on acid, high P-fixing soils and give almost the same results as applications of pure water soluble phosphate (CIAT, 1981). Chicken manure is sometimes used to improve the physical properties of the soil and to increase the P availability (Aidar *et al.*, 1976).

Besides reducing the Al toxicity, Ca from lime

Table 4. Nutrient composition in leaves and petioles taken from 3 different positions of the bean plant (*Phaseolus vulgaris* L.) grown on an oxisol of Brazil (unpublished data)

Position of the leaf in the plant		%					ppm					
		N	P	K	Ca	Mg	S	B	Zn	Fe	Me	Cu
Leaves												
Top	2nd & 3rd trifoliate	5.54	0.51	2.48	1.58	0.38	–	21.0	47	1139	72	19.7
Middle	intermediate	4.82	0.32	2.64	2.57	0.47	–	31.0	34	801	101	14.6
Lower	old leaves	3.42	0.21	2.28	4.04	0.54	–	35.0	27	3540	165	13.7
Petioles												
Top	2nd & 3rd trifoliate	2.52	0.24	3.96	1.34	0.24	–	34.0	33	497	35	10.1
Middle	intermediate	1.34	0.13	4.84	1.78	0.28	–	29.0	20	296	34	7.6
Lower	old leaves	1.40	0.14	6.37	1.99	0.29	–	37.0	25	1041	59	8.7

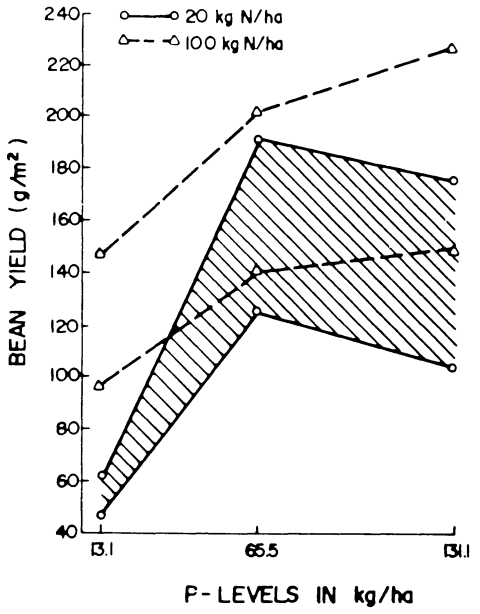

Fig. 6. Range of bean responses at different levels of P as affected by the level of N fertilization. (CIAT 1979).

improves P translocation in the plant (Pauli *et al.*, 1968) and the P absorption is enhanced by lime and N fertilization (Feitosa *et al.*, 1980). Mascarenhas *et al.* (1969) showed significant yield increases by liming and P fertilization. Feitosa *et al.* (1980) grew beans under irrigation and found linear yield increases at increasing P dosages in the presence of lime, similar results to those obtained by Fontes *et al.* (1965). Miyasake *et al.* (1966) showed in 7 experiments that lime produced a negative effect, only N and P increased yield. These depressive lime effects occured when the soil pH was above 5.8. In soils with a pH lower than 5.0, low P availability (1.5 ppm Bray II), and high in Al (2.0 meq/100 g soil), lime and P always increased yield.

In such soils, P fertilization higher than 88 kg P ha^{-1} will produce an average yield above 1 ton at any lime level. It is calculated that approximately 1 ton ha^{-1} of $CaCO_3$ equivalent of dolomitic lime and between 55–132 kg P ha^{-1}

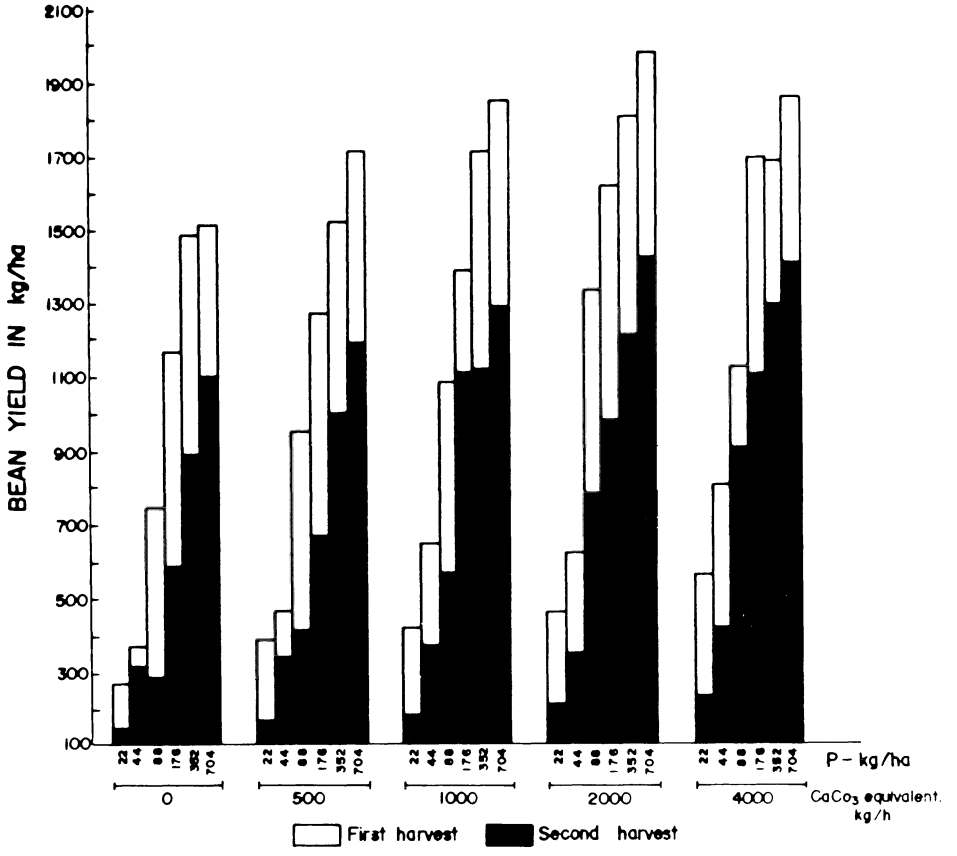

Fig. 7. Effects of different phosphorus and dolomitic lime levels on bean yields on two consecutive harvests. (CIAT 1982).

from water soluble P fertilizer are needed to produce 1000 kg bean ha^{-1}. Furthermore, there is a residual effect of about 66% in the second year of planting (CIAT, 1982). Figure 7 shows the yield differences between the first harvest and the second harvest, which did not receive additional P fertilization. It was also shown that an efficient bean line, "Carioca", outperformed the inefficient line, "ICA Tui", during both the first and second year (residual effect). This suggests that efficient lines yield better than in efficient lines at any level of P. Genotypic responses realized under high fertility (non-stress) conditions could be predicted reasonably well (Haag *et al.*, 1978).

The primary effect of amending the soil with organic matter is the improvement of the physical properties of the soil. The secondary effect of organic matter for the improvement of bean production on acid soils is that it reduces the Al toxicity effect at the same pH level and percent saturation rate (Bloom *et al.*, 1979); Evans and Kamprath, 1970). P-fixation on acid soils is also reduced by applying organic matter.

Green manure increases microbiological activity, which can temporarily immobilize N in the soil and consequently reduce the response to P fertilization. Miyasaka *et al.* (1967b) found that only N produced a response in N, P, and K experiments with and without green manure.

Differential responses of beans to P fertilization

Some cultivars grow well without P fertilizers in regions with different soil fertility levels, *e.g.*, "Carioca", "Rio Tibagi", "Ricopardo 896", G 5059", G 5054, and "Iguacu". Observations from farmers fields, later confirmed under greenhouse and field experiments, proved that there are differential responses of beans to different levels of P fertilization (Amaral, 1975; Lindgren, 1976; Rice, 1974; Salinas, 1978; Whiteakar *et al*,. 1976). Miranda and Lobato (1978) found different responses of beans to different levels of P, which they interpreted as too difficult to give a general P fertilizer recommendation. From these pieces of information, a screening methodology is established to select P-efficient cultivars and advanced breeding lines using yield as a parameter. The field method is simple and uses 2 levels of P (stress and non-stress levels). Response (alpha) is calculated as follows:

$$(\text{alpha}) = \frac{\text{yield under non-stress-yield under stress level}}{\text{difference of } P_2O_5 \text{ (kg ha}^{-1}\text{) applied between non-stress and stress level}}$$

The average yield of the stress plots of the experiment is used to separate the efficient and inefficient lines. The response average (mean of alpha) is used to separate the responsiveness of the lines tested. With this method we can classify bean cultivars into 4 groups (Fig. 8): efficient/responsive (quadrant I), efficient/non-responsive (quadrant II), inefficient/non-responsive (quadrant III), and inefficient/responsive (quadrant IV). Stress levels should be in accordance with local conditions. The response parameter is needed because bean cultivars should not only yield well under low soil P but also respond well to additional P fertilizer. The formula implies that alpha varies among the group to the disadvantage of the most efficient lines (those with highest yield under P stress conditions) (see quadrants I and II). These most efficient lines usually have a lower alpha value because the yield differences between the yield of non-stress and stress conditions are small and may fall into the quadrant II (efficient but non-responsive lines). These efficient, but non-responsive, lines are good for small farmers who are not used to applying fertilizer in their bean field and, therefore, should be taken into account in the evaluation process. To make the evaluation more applicable to different levels of agriculture technology another factor, APMY (*A*dditional *P*hosphorus needed to reach the *M*aximum *Y*ield) was introduced. This is the theoretical quantity of P fertilizer needed to raise the yield of these particular genotypes to equal the maximum yield of the experiment. For each genotype the following calculation is made:

$$\text{APMY} = \frac{\text{Maximum yield of the experiment} - \text{yield under P-stress}}{\text{alpha}}$$

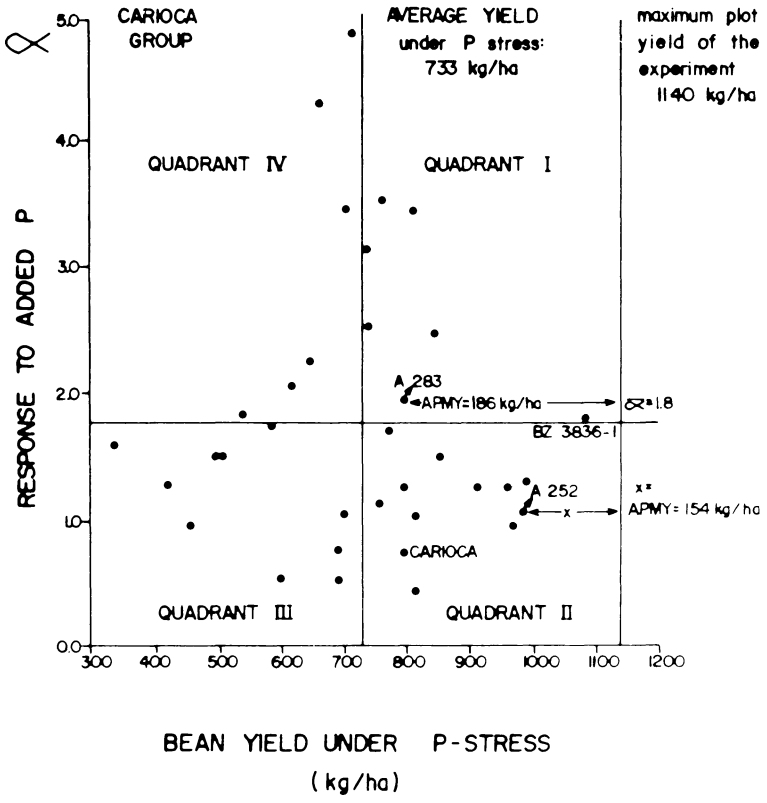

Fig. 8. Evaluation of bean according to their efficiency, response to additional phosphatic fertilizers, and the additional phosphatic fertilizer needed to reach the maximum yield (APMY).

and is expressed in P kg ha^{-1}. With this formula, the selection favors the most responsive lines (lines with high alpha values). A low APMY value means that only a small quantity of P is needed to reach maximum yield. Through this screening method, several lines and cultivars (Table 5) have been identified as efficient at low soil P. Results from this screening method confirmed the postulate of Lyness (1936), which stated: "Efficient lines under stress conditions may not be efficient on non-stress conditions and vice-versa".

Search for other selection parameters

Several experiments were conducted from 1979 to 1981 on a P-deficient soil at the CIAT-Quilichao station to find parameters suitable for discriminating efficient and inefficient lines. In summary, the results were:

1) Lines that utilize P efficiently yielded higher than inefficient lines when grown under P stress conditions (Fig. 9).

2) Inefficient lines produced higher leaf area indices than the efficient ones at 21 days but at flowering differences disappeared. The efficient lines produced higher LAI than the inefficient lines only at high P levels (Fig. 10).

3) Inefficient lines produced higher leaf weight than the efficient lines at 21 days after germination. At flowering time inefficient lines produced higher stem weight than the efficient lines but no difference occurred in leaf production (Fig. 11).

4) P content in the leaves of the efficient lines was slightly higher than in those of the inefficient ones at 21 days after emergence and the differences remained at flowering time.

Table 5. Outstanding advanced breeding lines and their checks with their response factors and additional phosphorus requirements to reach maximum yield

Identification	Yield kg ha^{-1} (14% hum.)		Response factor	Additional P requirement to reach maximum yield
	Non-stress plot	P-stress plot		
Carioca type				
BZ 3836-1	1275	1085	1.58	35
BZ 3836-2	1230	820	3.42	94
MX 2759-20	1210	720	4.08	103
BZ 3815-1	1190	765	3.54	106
A 251	1140	985	1.29	120
Checks				
A 252	1105	980	1.04	154
A 283	1020	800	1.83	186
Carioca	890	800	0.75	453
Cream seeded				
ESAL 506	1750	1070	5.67	44
82 PVBZ 1770	1400	1220	1.50	67
BZ 2231-11	1455	1080	3.12	77
82 PVBZ 1785	1480	820	5.50	91
Checks				
G 4000	1080	870	1.75	257
G 5054	1065	670	3.29	198
G 5059	870	750	1.00	570
Black seeded				
BAT 58	1405	1180	1.88	48
LM 30629	1450	825	5.21	85
CNF 480	1400	940	3.83	86
W 22-14	1385	935	3.75	89
LM 21135	1345	1045	2.50	90
LM 21132	1375	935	3.67	91
LM 21124	1345	1015	2.75	93
Checks				
Ica Pyao	1095	610	4.04	103
Puebla 152	900	810	0.77	613
Rio Tibaji	835	745	0.75	700
Purple seeded				
MX 1418-1	1755	1405	2.92	43
MX 2709-24	1510	1220	2.42	128
TY 3350-2	1415	940	3.96	149
TY 3364-9	1220	610	5.08	181
ESAL 503	1190	665	4.38	197
Checks				
BAT 41	830	720	0.92	880
CNF 10	510	460	0.42	2547

Table 6. Root length (cm) and relative root length (%) at five different depths of bean grown in Rhizoctron

Depth	Carioca		Rosinha 62		A 283		Puebla 152		CNF 10		A 143		TOTAL	
in cm	cm	%	cm	%	cm	%	cm	%	cm	%	cm	%	cm	%
0–20	143.5	40	147.4	43	135.2	60	125.1	43	83.0	64	52.3	48	686.5	47
21–40	102.1	28	119.5	35	65.5	29	73.8	25	38.5	30	56.2	5	455.6	31
41–60	73.7	21	49.4	14	23.9	10	87.7	30	7.5	6	0.8	–	243.0	17
61–81	38.8	10	16.5	5	–	–	8.7	3	–	–	–	–	64.0	4
81–1000	0.9	0	7.5	2	–	–	–	–	–	–	–	–	8.4	1
TOTAL	359.1		340.3		225.6		295.3		129.0		109.3		1457.5	

P content in the stems of efficient lines was higher than inefficient lines at flowering and at physiological maturity. No differences in P content were observed in pod walls or seeds (Fig. 12).

P contents in roots of efficient lines was equal to that of the inefficient lines at 21 days after germination and at flowering. However, at physiological maturity, the P content in the roots of inefficient lines was higher, especially when grown under low and very high rates of P fertilization (Fig. 13).

5) P absorption by the shoot of inefficient lines was higher than the absorption by efficient ones at 21 days after emergence. But, from flowering until maturity, P absorption of efficient lines was higher (Fig. 14).

The P absorption curve by the bean root was

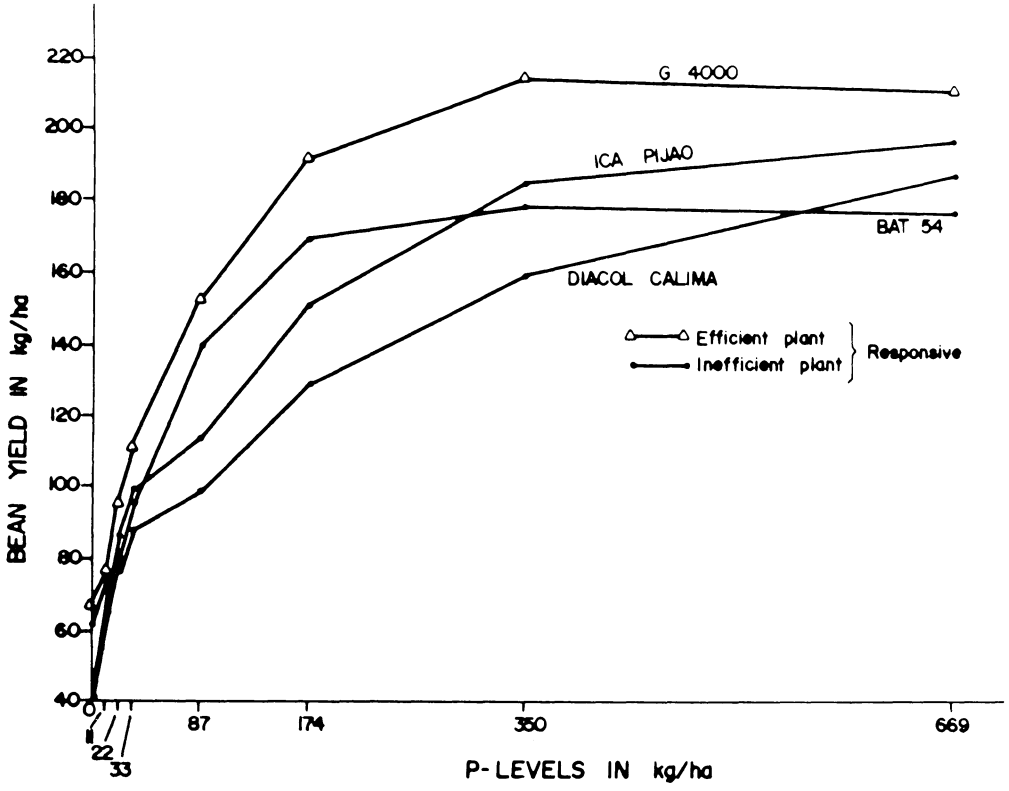

Fig. 9. Yield of efficient and inefficient plants grown under eight P-levels. (CIAT 1980).

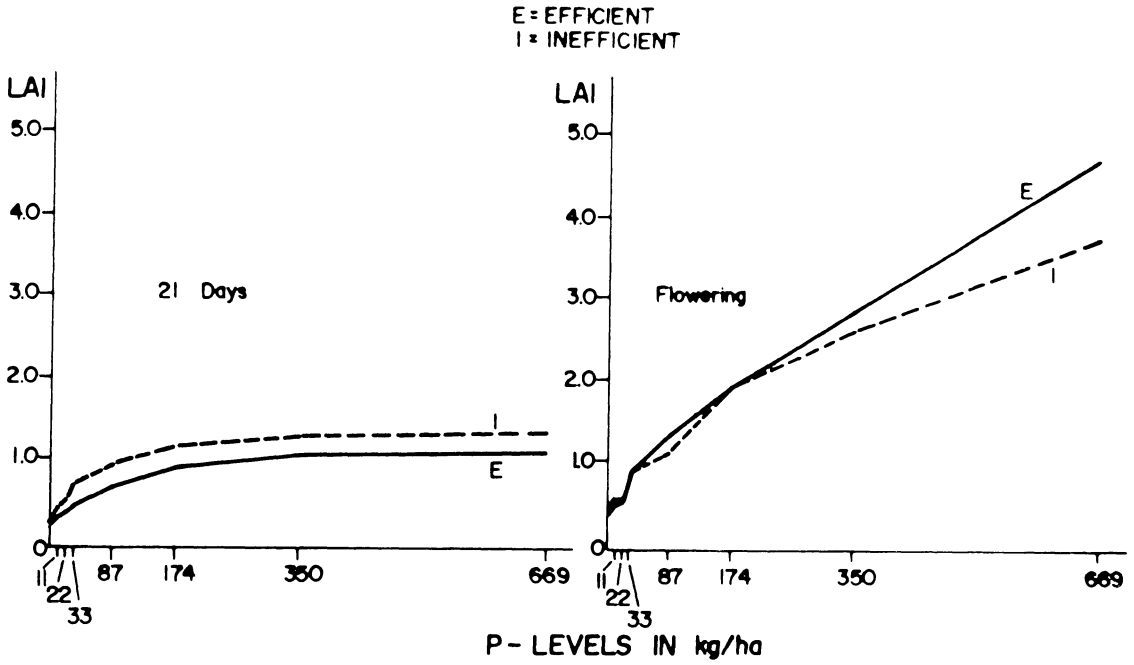

Fig. 10. Leaf area index (LAI) of an efficient and inefficient plant in different P-levels at 21 days after germination and at flowering.

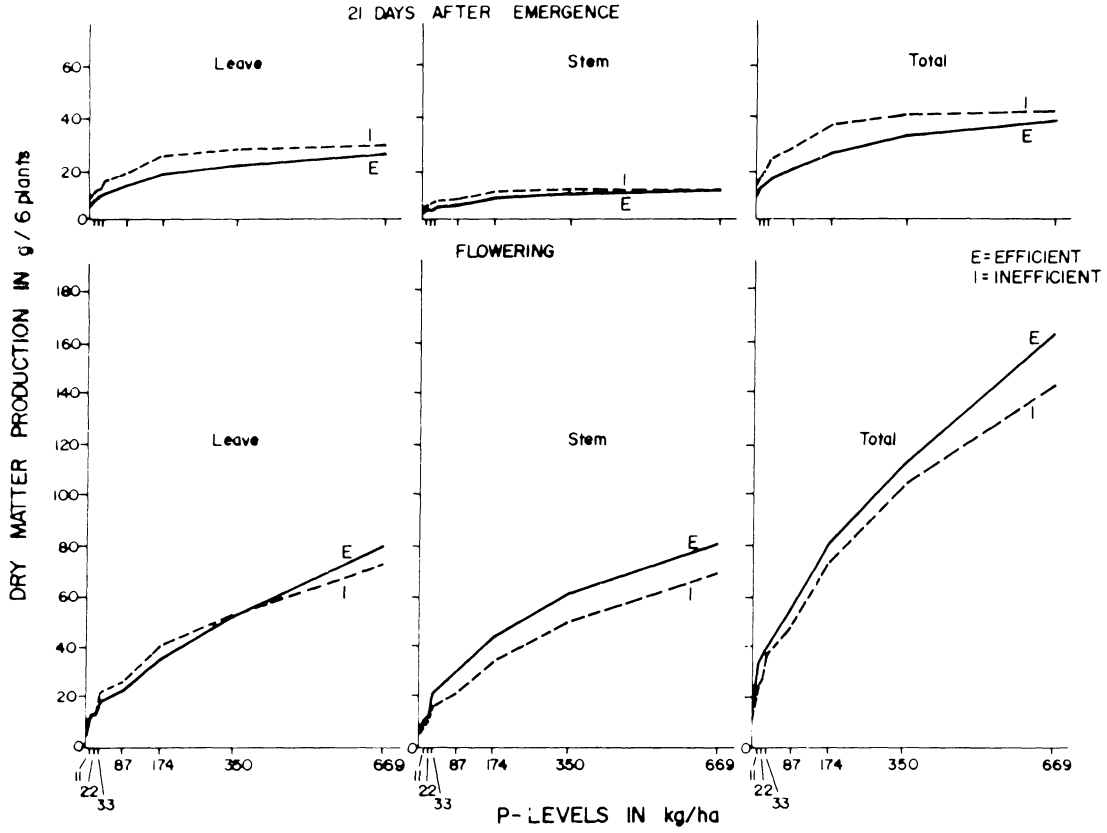

Fig. 11. Leaf and stem weight of efficient and inefficient plants in different P-levels at 21 days after germination and flowering.

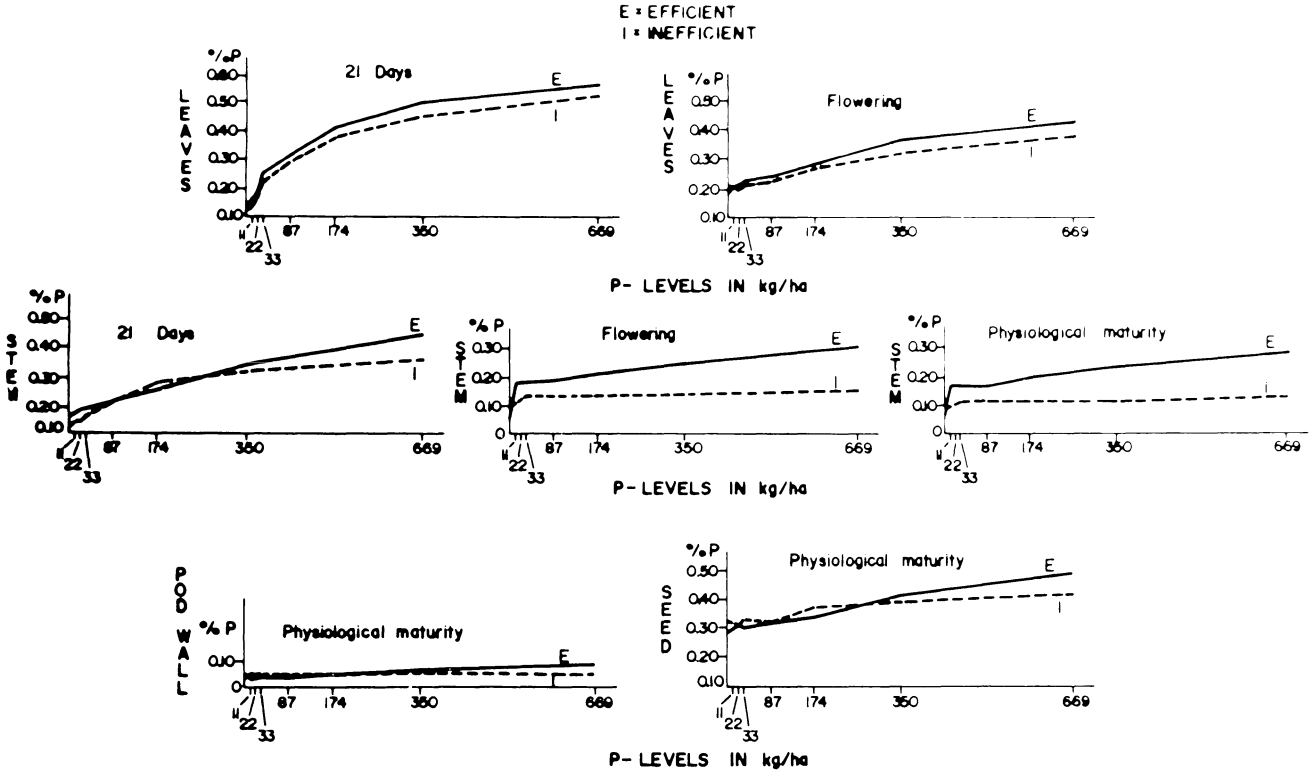

Fig. 12. P content (%) of efficient and inefficient plants in different plant parts at different growth stages.

similar to that of the bean shoot except at physiological maturity wehn inefficient lines showed higher P absorption rates than efficient lines (Fig. 15).

The efficiency with which beans utilize soil P is controlled genetically, and efficiency apparently lies in P recycling and turnover to produce grain (Amaral, 1975).

Guadron (1985) conducted experiments with several combinations of split applications from 21 days after germination to post-flowering to avoid P fixation by an acid soil. Inefficient lines, even with a continuous supply of P, still did not outperform the efficient ones (Guadron, 1985).

Screening for low soil P efficiency at CIAT Quilichao did not progress rapidly because of inconsistencies over cycles of testing, especially for those lines that were not too distinct in their efficiency or responsiveness (they lie too close to the divisional lines of average yield under stress conditions and average alpha values). Furthermore, selection by visual observation during the early stages of growth also proved to be difficult because the inefficient lines look more vigorous than the efficient ones. These problems were circumvented by testing over several seasons with increasing stress levels. Several researchers attempted to screen for low soil P efficiency under controlled conditions (Rice, 1974; Salina, 1978; Whiteaker *et al.*, 1976). Inconsistencies were caused by a number of problems including seed quality, lack of uniformity in seed and soil, and using other parameters rather than seed yield.

Some potential parameters for screening for low soil P efficiency

Shoot

As mentioned before, dry matter production in early growth stages is not a good criterion for selection. Some inefficient lines produce more

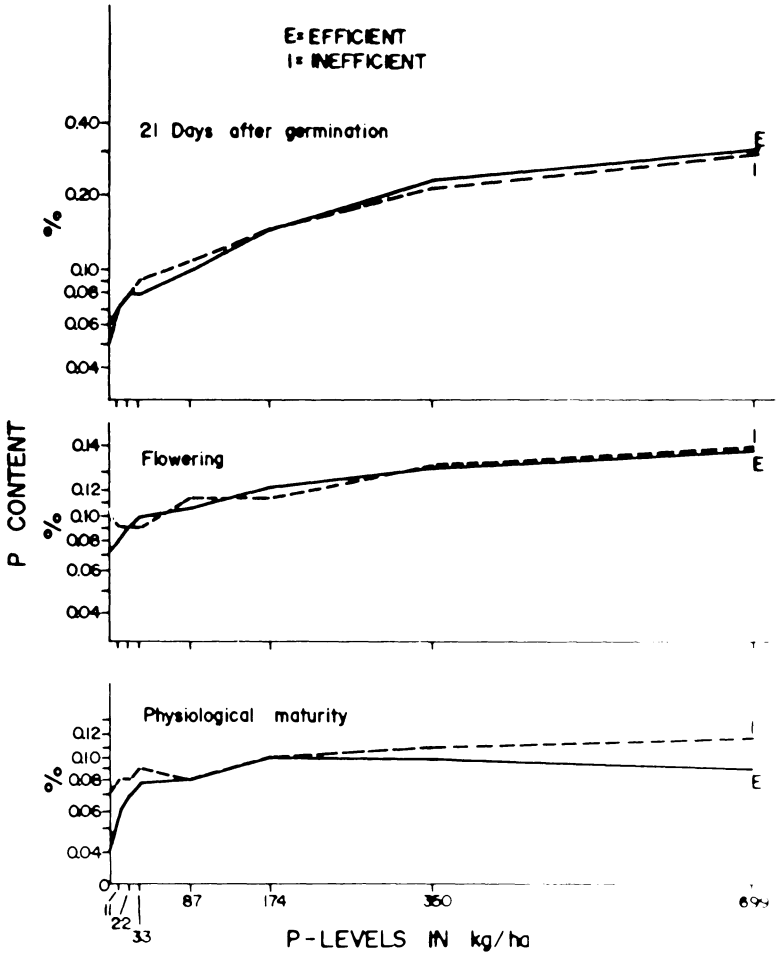

Fig. 13. P content in bean roots at different growth stages.

dry matter than efficient ones in the early growth stages. These results were again confirmed at CIAT (1982). Accurate visual evaluation in this case is impossible. P content is a highly variable parameter and is influenced by edaphic and climatic conditions (Barbosa, 1977; Terman *et al.*, 1967). Also, there is no significant difference in P content between efficient and inefficient lines (CIAT, 1982). Furthermore, P content depends not only on the plant age (Barbosa, 1977), but also on the plant organ and its position in the plant. Leaves and petioles from different heights on the plant differ in their P content (Table 4). P content is higher in the younger part of the plant than in the older leaflets and petioles. In addition, determination of the total tissue P content does not distinguish between organic and inorganic forms. The inorganic P found in the vacuole or in the xylem is not involved in metabolic processes. Plants with a high P content (P accumulators) cannot be assumed to be efficient plants. Total P does not indicate how much is being utilized in metabolic processes and how much remains idle in the vacuole (although P stored in the vacuole may be utilized during stress periods). Therefore, total P content is not a suitable selection criterion. The composition of other nutrients in the tissue and their relation to P content should be investigated, as Duncan (1982) proposed in selecting sorghum for acid soil tolerance. Butler *et al.* (1962) estimated the heritability of the major constituents of ryegrass vegative matter. No significant correlation was found between top growth and any of the miner-

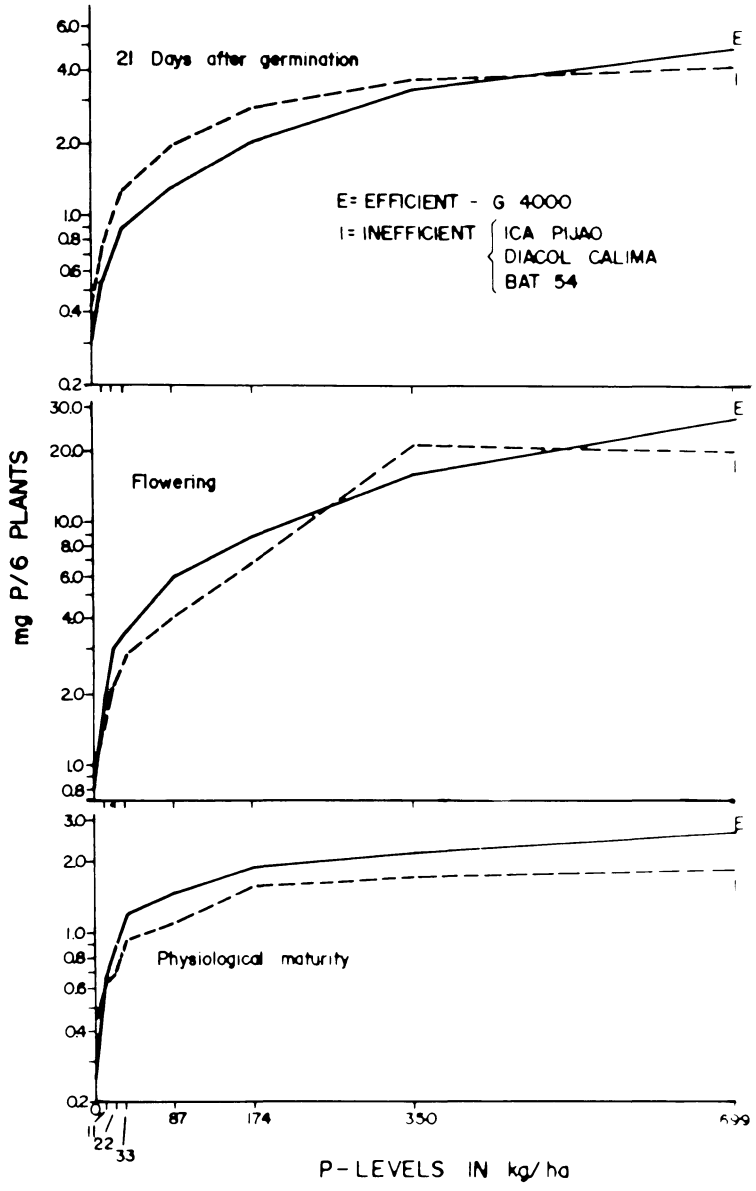

Fig. 14. Phosphorus absorption in mg P/6 plants by the aerial part of the bean plant at different growth stages.

al constitutents, which suggests that selection for dry matter production and P content could be independent, as Lindgren (1976) found in beans. Baker *et al.* (1964) found that P content in corn was not significantly correlated with any other nutrient or with yield.

Employing an all-possible diallele analysis, Gorsline *et al.* (1964) determined the mode of inheritance of P accumulation in leaves and grain of maize. Additive gene action was more important than nonadditive action in determining P accumulation. It is suggested that there may be different genes controlling P accumulation in leaves and in grain. Baker *et al.* (1964) found that the accumulation capacity of the plant does not distinguish between ions. Kleese *et al.* (1968) found that accumulation of one ion is not accompanied by accumulation of others, suggesting differential accumulation from independent mechanisms controlled by different genes. Tolerance to high levels of P, and no yield depression under high P are maternally inherited in beans

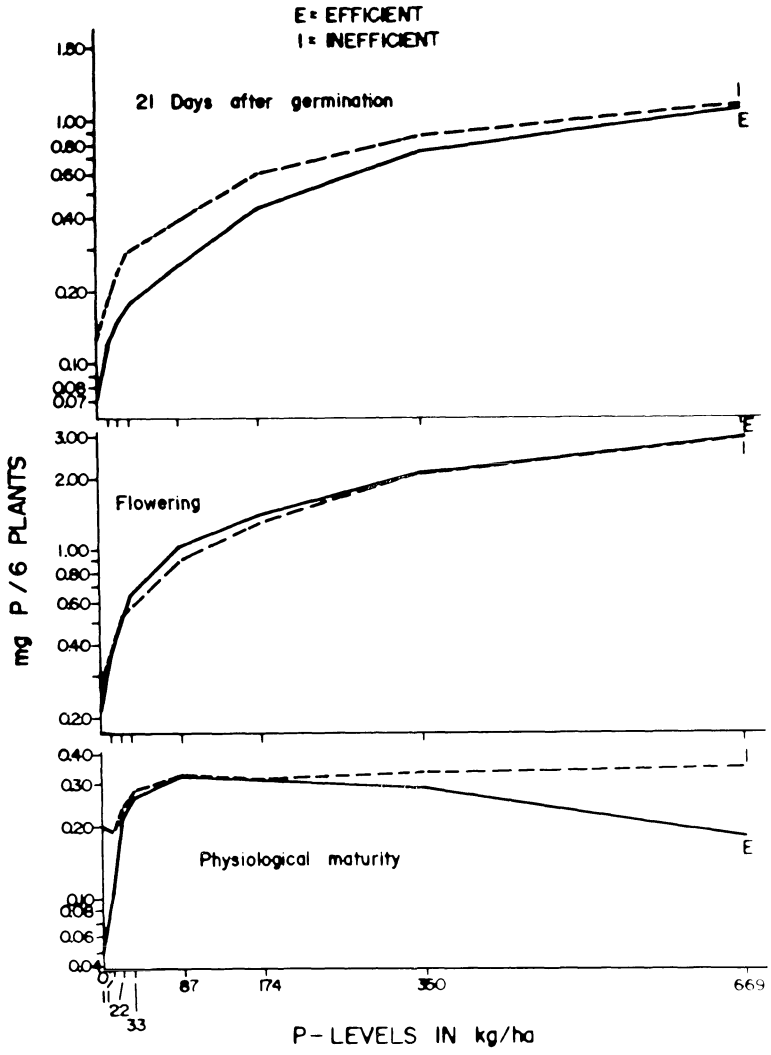

Fig. 15. Phosphorus absorption in Mg P6 plants by extractable root at different growth stages.

(Rice, 1974). P transfer within the plant is an active process (Epstein, 1972). Plants under non-stress conditions release more ions to the xylem, where they are upwardly transported to the active, terminal parts of the plant. An efficient plant will translocate mobile elements, such as P, from the older plant parts, where metabolic activity is low, to the young terminal parts.

Root

Root growth is reduced not only by Al toxicity and Ca deficiency, but also by P deficiency. The root system functions to absorb nutrients (including P) from the soil solution, and has the potential to take up phosphate faster than it can be supplied by the soil solution (Shapiro *et al.*, 1960).

The degree of division of a root system is important in determining the plant's ability for soil exploration and contact with soil P. Lyness (1936) determined that the overall structure of the root system was closely correlated with the ability for P uptake, and Troughton (1968) suggested that differences in P utilization attributed to root system differences may be related to shoot growth. However, shoot growth of the bean plant did not correlate with efficiency of P utilization or with yield under stress conditions. Baker *et al.* (1970) found no correlation between

differences in P uptake and root parameters such as length, diameter, and number of seminal roots in hybrid maize. Lewis and Quirk (1967) have shown that 85 percent of plant P is taken up by the root hairs. Nye (1966) concluded that thin roots covered with root hairs would be the most efficient root system (per unit mass) for absorption of slowly diffusing ions such as phosphate. Fawole *et al.* (1982) studied the genetic control of root development in 6 bean lines grown under P stress and observed that there were quantitative inheritance patterns and transgressive segregation for root dry weight. High, broad sense heritability estimates were obtained. Root/shoot relations are also unstable because they are influenced by genotypic variability in the response to environment (Taylor, 1981). Troughton (1968) found that increases in shoot growth were accompanied by smaller increases in root growth, suggesting that genotypes with rapid rates of shoot growth should have the most efficient root system in terms of mineral absorption per unit weight. Bloom *et al.* (1985) analyzed plant growth with economic theorems in allocation of resources at different environmental conditions and concluded that there was an optimum root:shoot ratio under given environmental conditions. In drought stress studies on 500 accessions from the CIAT germplasm bank, Hidalgo (1977) classified beans into 4 root types, 1) thick main central root, 2) thin main central root, 3) only secondary roots and 4) only fibrous root, but did not determine the volume of soil explored by each root type.

Soil microorganisms greatly influence P uptake. Bacteria, fungi and other microflora may enhance P uptake to some extent. The importance of root mycorrhiza in increasing P uptake and alleviating deficiency symptoms is well documented (Bieleski, 1973; Mosse, 1973). Larger absorption surfaces are created by the mycorrhizal hyphae, enabling the plant to explore a greater volume of soil than with the roots along, but mycorrhiza do not always exploit insoluble forms of phosphate. Also, Mosse (1973) indicated that host specificity exists in mycorrhizae and that some strains are more beneficial than others in P deficient soils. It appears that in this case there is also genetic control of host/symbiont specificity, about which little is known. Ferreira (1987) showed that mycorrihizae are indispensible to establish and increase the number of nodules in the bean plant. Cultivars with large CECs would have a potential for greater accumulation of cations. Varietal differences in CEC have been reported in ryegrass (Mouat, 1962), and legumes have twice the CEC of grasses (Vose, 1963). Butler *et al.* (1962) reported that positive correlations exist between CEC and cation accumulation in ryegrass. Concomitantly, significant negative correlations were found between anion content (especially phosphate and nitrate) and root CEC. Thus, selection for high CEC may reduce P uptake.

Biochemical processes

P-deficiency tends to increase cellular levels of acid phosphatases (CIAT, 1985). This enzymes may leak into the cell walls and into the surrounding medium where inorganic P may be released by hydrolysis of phosphate esters. The activity of acid phosphatase in the unsevered root, as well as in the leaf (disks), was lower in some efficient lines than in inefficient lines (CIAT, 1985). Further studies with more lines are still needed to confirm these results. It is not yet clear whether the role of these enzymes is to release inorganic P from soil organic P or to act as a trap for P esters leaking out of the root, converting their organic P into reabsorbable forms (Clarkson, 1985). The efficiency of the metabolic process is another process which may be of interest. Whiteaker *et al.* (1976) indicated that at the same P concentration in bean leaves, the photosynthetic rate was higher in P efficient than in P-inefficient plants when both were grown under P stress. But, the measurement of P concentration in the leaves did not separate the organic and inorganic P forms. The latter is not utilized in metabolism but remains in the vacuoles (Bieleski, 1973). The heritability of efficiency in P utilization was studied by Fawole *et al.* (1980) using the estimates of broad sense heritability. The results showed that efficiency in P utilization is a highly heritable trait (where efficiency is defined as the ratio of plant dry weight produced/mg P absorbed by the plant).

Conclusions

Screening for low soil P-efficiency in bean concentrated heavily on the response of the bred lines and breeding for efficiency to low soil P was not very successful because of selection criteria which relied heavily on visual evaluations and yield; but yield unfortunately cannot be predicted from bean grown under stres conditions (Haag *et al.* 1978).

An integrated plant nutrient and plant breeding approach directed towards a better bean plant adapted to acid soil is being elaborated. From information obtained from the field experiments, breeding can start combining any traits that may enhance the absorption, transport, and utilization of P by the bean plant *e.g.*:

1. Improving P absorption ability by improving root formation and using as parent ICA COL 10103, Rosinha G2, and HF 465-63-1 which have deep roots under field conditions. Fawole *et al.* (1982) showed that under controlled conditions the root has a quantitative inheritance pattern where dominance variance was more important than additive variance.
2. Improving P uptake and accumulation in early growth to confront the stress situation later in the crop cycle. This mechanism will only work providing that the translocation ability of the plant is high enough to translocate the P in the plant to the active part. High accumulation of P in early growth is detected in Puebla 152 (CIAT, 1987).
3. Improving P transport from the root to the shoot. Inefficient lines tend to accumulate P in the root. Selection with low P content in the root may indicate a better transport. Cultivars that have low P accumulation in the root are: G 4000 (CIAT 1979) and Carioca (Urea, 1984).

Other parameters which still cannot be used by breeders because of lack of practical methods are: 1. Better mobilization of P from older parts which will soon be senescent to younger short parts where active metabolic processes take place; 2. Better utilization or translocation of accumulated P in vacuoles during the stress period; 3. Higher release of acid phosphatase enzyme by the root; 4. More efficiency in producing biomass per unit P and; 5. Better symbiosis with V.A. Mycorrhize.

Furthermore, breeding for P efficient use, when it can be successfully adopted by the farmer, must give higher yield than the existing local or recommended variety, have resistance to prevalent production constraints such as disease and insects where common bean mosaic virus is a must for Latin America and, have commercial grain type accepted by the farmers.

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PLSO PN 194

Screening of rices for adverse soil tolerance

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Key words: minerals, rice, soil tolerance

Abstract

To increase rice yields on adverse soils without costly soil amendments and reclamation, rice cultivars are needed that tolerate the various associated mineral stresses. In South and Southeast Asia, salinity, alkalinity, strong acidity and excess iron hamper rice growth on about 5 million ha. Because of soil problems 23 million ha of land suited to rice lie idle. Screening methods for the various chemical soil stresses have enabled the development of higher yielding cultivars with increased tolerance to adverse soil conditions. Yield advantage of up to 3 t/ha over traditional varieties have been demonstrated in farmers' fields. The multiple soil stress tolerance of the cultivar IR36 has contributed to its cultivation on almost 11 million ha worldwide.

However, lack of knowledge on the mechanism and genetics of tolerance constrains a more rapid and consistent development of adverse soil tolerant rice cultivars.

Introduction

The development of rice cultivars with greater tolerance to adverse soil conditions offers considerable promise for increasing rice production on marginal and less fertile lands. Rice cultivars tolerant to soil stresses are of particular help to small farmers who cannot afford costly amendments or high fertilizer inputs. Toxicities hamper wetland rice growth on saline, alkaline, acid sulfate and peat soils and upland rice on acid soils. Nutrient deficiencies prevent modern high yielding rice cultivars from expressing their yield potential in many soils. Zinc deficiency is the most widespread micronutrient deficiency in wetland rice soils. P deficiency is common in acid and alkaline wetland rice soils and in most uplands grown to rice. In South and Southeast Asia about 23 m ha of idle land are suited to adverse soil-tolerant rices (Boje-Klein, 1986).

This paper discusses the impact of screening at the International Rice Research Institute (IRRI) on the development of high yielding rice cultivars with increased tolerance to adverse soil conditions.

Chemical soil stresses

The soil representing nutritional environments of extraordinary spatial and temporal diversity is an important determinant of plant growth. It is probably the most heterogeneous compartment. The nature of soils is characterized by complex and intimate interaction of all soil components. Therefore a soil stress rarely occurs in isolation. Soils such as saline, sodic, acid sulfate and peat soils are characterized by an array of chemical stresses (Table 1). The chemistry of adverse soil conditions and nutrient disorders in rice soils have been discussed (Ikehashi and Ponnamperuma, 1978; Neue and Singh, 1984; Neue and Zhu Zhonglin, 1989; Ponnamperuma, 1985).

It would be possible to take into account all soil variables in a breeding program. A breeding strategy does not only have to increase tolerance to soil stresses but has to include other plant characteristics like yield potential, grain quality, insect and disease resistance, plant type and growth duration. Therefore we have amalgamated the various soil stresses into broader groups and focused

Table 1. Problem soils and associated stresses

	Saline	Saline/ sodic	Saline acid	Acid sulfate	Peat
Acidity	–	–	×	×	×
EC > 4 dS/m	×	×	×	–	–
Low base status	–	–	×	×	×
P deficiency	×	×	×	×	×
Zn deficiency	×	×	–	–	×
K deficiency	×	–	–	–	×
Si deficiency	–	–	–	–	×
Fe deficiency	–	×	–	–	×
Na toxicity	×	×	×	–	–
Fe toxicity	–	–	×	×	×
Mn toxicity	–	–	×	–	–
Al toxicity	–	–	×	×	–
B toxicity	–	×	–	–	–
H_2S toxicity	–	×	–	–	×
Toxicity of organic substances	–	–	–	–	×

Adapted from Neue and Singh, 1984.

Table 2. Summary of screening for chemical stress tolerance, IRRI 1969–1988

Stress	Entries tested (No.)	Entries found tolerant	
		(No.)	%
Salinity	110,274	20,945	19
Alkalinity	26,692	4,275	16
Peat soil	2,855	282	10
Fe toxicity	6,320	490	7.8
P deficiency	10,149	1,318	13
Zn deficiency	23,202	1,917	8.3
Fe deficiency	891	85	9.5
Al or Mn toxicity	2,055	222	11
B toxicity	644	140	21
Total	183,102	29,674	16

screening on traits that are most widespread. The summary of screening at IRRI (Table 2) reflects the emphasis put on the different soil stresses.

Identification of soil stresses in rice

Visible scoring of chemical soil stresses is still an indispensible method of stress identification. Visual diagnosis is fast and does not require special equipment. The visual symptoms arising from chemical soil stresses in rice and related scoring are given elsewhere (IRTP 1988).

To make scoring data meaningful the stress conditions and the growth stage of plants have to be defined in detail. We have established standard soil stresses (Table 3) at which most rices will survive but can be discriminated according to their tolerance level. Rices are rated tolerant (score 1 — 3) if the imposed stress does not severely retard growth manifested by visual symptoms. The normal distribution of the frequency of scores collected in the greenhouse and field (Fig. 1) reveal that the discriminating levels of soil stresses are properly defined. The frequency distribution of scores collected in selected fields with similar stresses differ only slightly. Since deficiency or toxicity symptoms clearly appear unspoiled at early growth stages scoring is done 4 or 8 weeks after transplanting (WAT) 2 weeks old seedlings raised in normal culture solution. Yield trials revealed significant correlations with tolerance scores. Relative grain yield was highest in those rices with low scores.

The established screening techniques are rapid, reliable, and suited to large numbers of cultivars.

To confirm results from mass screening, promising rices from IRRI and national breeding programs are tested further in saline environments in different countries under the International Rice Testing Program (IRTP). Performance and yield trials are also done with best performing cultivars.

Adverse soil tolerance

Salinity is the main obstacle to high rice yields in coastal areas in the humid tropics and in arid and semiarid areas where evaporation exceeds precipitation. Saline soils are those that have an electric conductivity in the saturation extract (ECe) exceeding 4 dS/m at 25°C (US Salinity Laboratory Staff 1954) but vary in their chemical, physical and hydrological properties. Saline soils vary widely in their chemical and physical properties. The pH ranges from extremely acid in saline acid sulfate soils to alkali in saline sodic soils, organic matter content from very low to peaty; and nutrient status is varied (CSSRI, 1979; Ponnamperuma and Bandyopadhyay, 1980; van Breemen, 1976).

If compared with other crops rice is moderately sensitive to salt stresses (Maas and Hoffman, 1977). But rice is highly suited to large areas of saline soils because it tolerates standing water, which is necessary for reclamation. Flooding saline soils with fresh water during the rainy season or with irrigation

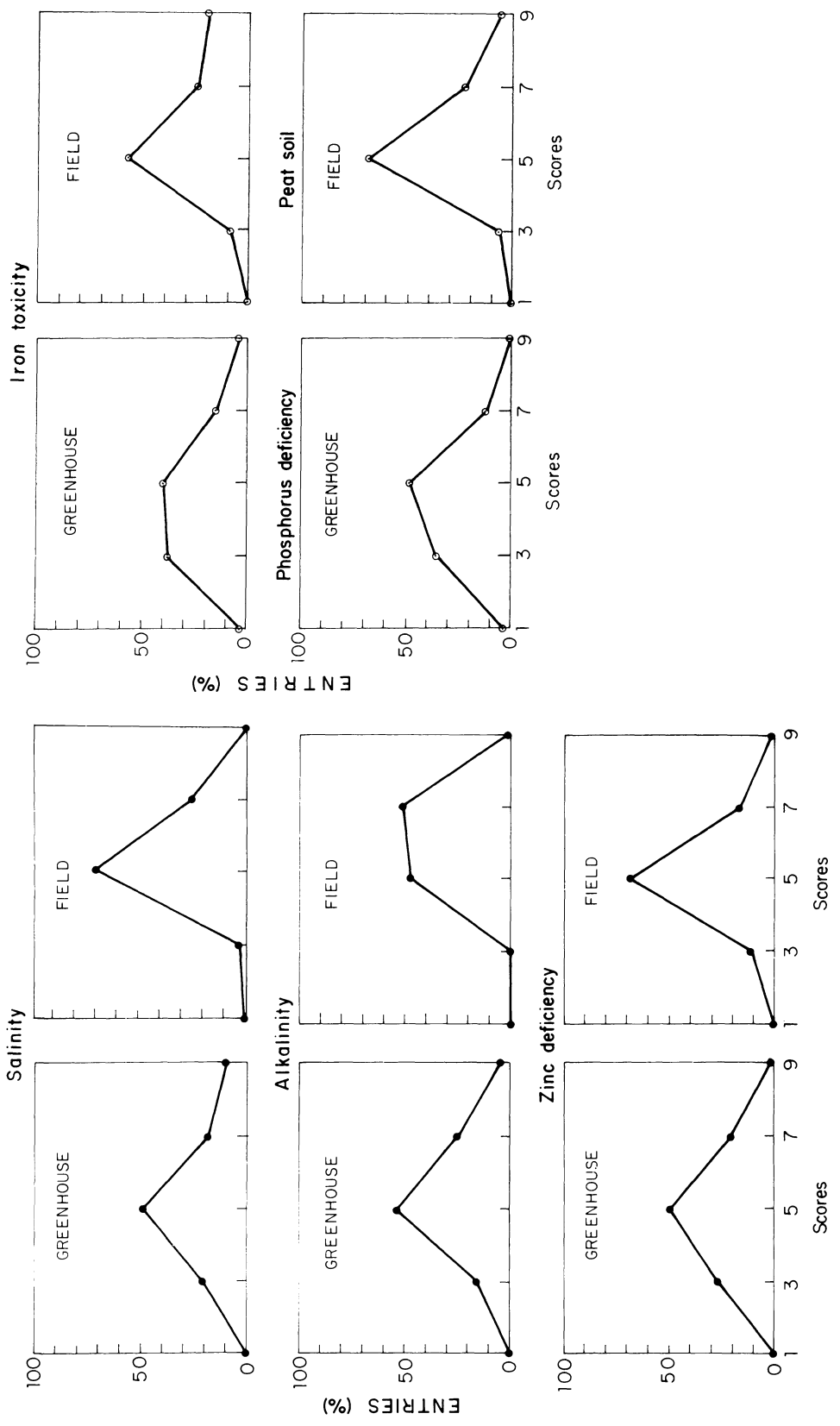

Fig. 1. Frequency distribution of adverse soil tolerance scores.

Table 3. Screening procedures for adverse soils

Stress	Soils/ medium	Fertilizers	Method of planting	Layout	Check varieties	Rating time (SES)[a]
Salinity	Maahas clay treated with common salt to an EC of 8 dS/m	25 mg kg N^{-1} as urea	Transplant 2-week-old seedlings grown in culture solution	Greenhouse Trays, 35 × 27 × 11 cm 3 seedlings/variety 3 varieties/tray	T: IR9764-45-2-2 S: MI-48 T and S checks in each tray	4 WAT
	Maahas clay treated with common salt to an EC of 8 dS/m	100 kg N ha^{-1} as urea 20 kg P ha^{-1} as solophos	Transplant one month old seedling grown on wet seedbed	Block L5 IRRI Farm 2.1 m rows, 20 × 25 cm	T: IR9764-45-2-2 S: IR5929-12-3 T and S checks after every 18 entries	4 and 8 WAT
	Maahas clay treated with common salt to an EC of 8 dS/m	100 kg N ha^{-1} as urea	Transplanting one month old seedling grown on wet seedbed	Block 428, IRRI Farm Three 5 m rows, 20 × 20 cm, 3 reps	T: IR9764-45-2-2 IR9884-54-3 S: IR5929-12-3 T and S check after every 10 entries	4 and 8 WAT
Alkalinity	Maahas clay treated with Na_2CO_3 to obtain a pH of 8.6 SAR: 35	25 mg kg N^{-1} as urea 4% zinc oxide dip	Transplant 2-week-old seedling grown on culture solution	Greenhouse Trays, 35 × 26 × 11 cm 3 seedlings/variety 3 varieties/tray	T: IR46 S: IR5931-110-1 T and S checks in each tray	4 WAT
	Maahas clay treated with Na_2CO_3 to obtain a pH of 8.6	100 kg N ha^{-1} as urea 20 kg P ha^{-1} as solophos 4% zinc oxide di	Transplant one month old seedling raised on wet seedbed	Block L5, IRRI Farm 3.1 m rows at 20 × 15 cm	T: IR46 S: IR5931-110-1 T and S checks after every 10 entries	4 WAT
	Maahas clay treated with Na_2CO_3 to obtain a pH of 8.6	100 kg N ha^{-1} as urea 4% zinc oxide dip	Transplant one month seedlings raised on wet seedbed	Block 429, IRRI Farm Three 5 m rows, 0.6 × 5 m per entry 20 × 20 cm, 3 reps	T: IR46 S: IR5931-110-1 T and S checks after every 10 entries	4 WAT
Peat soil problems	Calauan, pH: 5.8 Org. carbon: 25.6%	50 kg N ha^{-1} as urea 25 kg P ha^{-1} as single super-phosphate 50 kg K ha^{-1} as muriate of potash	Transplant 3-week-old seedlings raised on same soil	Three 5 m rows, 20 × 20 cm 3 reps	T: IR8192-31-2-1-2 S: IR5931-110-1 T and S checks after every 20 entries	4 and 8 WAT

Acid sulfate soil conditions/ iron toxicity	Balza, Malinao, Albay: pH 3.7, Org. carbon: 0.6% Active Fe: 2.3%, Active Mn: 0.003%	50 kg N ha^{-1} as urea 25 kg P ha^{-1} as solophos 25 kg K ha^{-1} as muriate of potash	Transplant 3-week-old seedlings raised on same soil	Three 5 m rows, 20 × 20 m, 3 reps	T: IR36 S: IR21015-136-1-2-3-1 T and S checks after every 10 entries	4 and 8 WAT
Zinc deficiency	Bay, pH: 7.2, Org. carbon: 3.9% Avail Zn: 0.19 ppm	No fertilizer	Transplant 3-week-old seedlings grown in trays in greenhouse T and S after every 10 entries	Three 5 m rows, 20 × 20 cm, 2 reps	T: IR34 S: IR26 and IR6115-1-1	4 WAT
Phosphorus deficiency	Culture solution	P levels, 0.5 ppm and 10 ppm	Direct seed	Greenhouse 3-L pots 4 seedlings/variety 1 variety/pot 2 reps	T: IR54 S: IR6115-1-1 T and S check after every 50 entries	4 WAT
Boron toxicity	Maahas clay treated with 10 mg B kg^{-1} as borax	50 mg kg^{-1} as urea 25 mg kg^{-1} as superphosphate	Transplant 2-week-old seedlings grown on culture solution	Greenhouse 16-L pots 4 seedlings/variety 1 variety/pot 2 reps	T: IR9129-209-2-2-3 S: IR29723-143-3-2-1 R and S checks after every 20 entries	8 WAT and maturity

[a] Standard Evaluation System. T = tolerant; S = susceptible, WAT = weeks after transplanting, SAR = sodium adsorption ratio.

Table 4. IRRI lines released as varieties due specifically to their tolerance to specific soil stress

Year	IRRI lines	Variety name given	Country where named	Soil stress
1980	IR2823-399-5-6	NN2B	Vietnam	Adverse condition in acid sulfate soil
1981	IR1416-131-5	Suakoko 12	Liberia	Fe toxicity
	IR2071-625-1-252	IR36	Philippines India	Multiple stress
1982	IR2071-586-5-6-3	IR42	Philippines Indonesia Malaysia Kampuchea Nigeria	Multiple stress
	IR4570-83-3-3	NN5B	Vietnam	Acid sulfate
1985	IR5657-33-2-2-3	Citanduy	Indonesia	Salinity
1987	IR8192-200-3-3-1	Lemaya	Malaysia	Fe toxicity
	IR2151-196-3-1-3	IR2151	Vietnam	Saline acid sulfate
	IR2153-26-3-5-6	IR2153	Vietnam	

water lowers salinity levels so that tolerant cultivars could be grown on 6.3 million ha in South and Southeast Asia alone (Boje-Klein, 1986).

A greenhouse technique suited for a large number of entries was devised (Ponnamperuma, 1977) using soil culture at the discriminating salinity level of $8\,dS\,m^{-1}$ (Table 3). Materials identified as tolerant have been successfully used as parents in hybridization programs at IRRI and in national programs (Tables 4 and 5).

Recent studies have identified the mechanism of salinity tolerance in rice (Akita and Cabuslay, 1989; Yeo and Flowers, 1982; 1983; 1984; Yeo *et al.*, 1988). Salinity stress is predominantly due to excessive Na ion uptake and Na accumulation in the leaves. Important morphophysiological variables that affect salt tolerance in rice are restricted Na uptake, retention in roots, compartmentation in stem, leaf sheath and older leaves, dilution through rapid growth, and increased K-uptake. Tissue tolerance seems to be of minor importance (Zapata and Abrigo, 1986).

The reported mechanism clearly indicate that salinity is a multivariable phenomenon and tolerant traits may not be expressed in the phenotype. Increased yield potential and salinity tolerance can be combined if tolerance is not at variance with the desired agronomic characteristics. Rapid and reliable screening methods to identify the desired traits in cultivars have still to be developed.

Alkalinity

About 1.5 million ha of rice land in India and Pakistan are affected by alkalinity. About 4.2 million ha of alkaline soils are suited to rice if tolerant cultivars are available. Alkali soils contain sufficient exchangeable sodium to depress plant growth. The sodium adsorption ratio (SAR) of the saturation extract exceeds 15 and the pH is usually above 8.5. FAO/UNESCO (1973) grouped rice as moderately tolerant to alkalinity. Most of the growth limiting factors are alleviated by flooding but Zn and P deficiencies remain severe.

About 16% of rices tested have been found tolerant. Though the proportion of the total variation accounted for is small alkalinity tolerance is significantly correlated with salinity ($r = 0.15^*$) and P deficiency tolerance ($r = 0.17^*$).

Because of the need for good grain quality (Pakistan) and widespread soil reclamation of alkaline soils in India through application of gypsum or iron sulfate the release and acceptance of tolerant cultivars has been limited.

Acid sulfate soils

Rice is grown on about 1 million ha in Vietnam. The potential area for tolerant rice cultivars amounts to about 3 million ha in South and Southeast Asia (Boje-Klein, 1986). Acid sulfate

Table 5. Adverse soil-tolerant rice cultivars* developed in national programs

Cultivars	Country of origin
Salinity	
A69-1	Sri Lanka
RP975-109-2	India
BR 51-282-5	Bangladesh
B2433-b-Kn-10-1-1-1	Indonesia
C100	India
C2	India
ROK 5	Sierra Leone
PNL 5-1-1-1	India
PNL 36-184-3-2	India
PNL 5-30	India
Alkalinity	
RP 975-109-2	India
BR 51-282-8	Bangladesh
ROK 5	Sierra Leone
Acid Upland	
IRAT 144	Ivory Coast
IRAT 104	Ivory Coast
UPL Ri-5	Philippines
UPR 103-80-1-2	India
M55	Liberia
GA 305	Indonesia
ITA 142	Nigeria
ITA 235	Nigeria
ITA 116	Nigeria
BG 35-2	Sri Lanka
BPI Ri-6	Philippines
B733C-167-3-2	Indonesia
Acid Lowland	
BR51-120-2	Bangladesh
BW 267-3	Sri Lanka
BG 374-1	Sri Lanka
BW 271-1	Sri Lanka
P1369-4-16M	Colombia
MRC 172-9	Philippines
BW267-3	Sri Lanka
B2149b-Pn-26-1	Indonesia
MRC 172-9	Philippines
P1274-6-8M-1-3M	Colombia
P1369-4-16M-1-2M	Colombia
P1391-6-11M	Colombia

* From Nursery Trials of the International Rice Testing Program (IRTP) 1982–1987, IRRI, Manila, Philippines.

soils are characterized by a pH < 4.0 due to sulfidic/sulfuric materials. Though flooding alleviates some growth limiting factors, iron toxicity and P deficiency remain as serious problems.

Both mineral stresses are also common on highly weathered acid wetlands, for example, in Sri Lanka, Africa and South America. Iron toxicity is often induced by a multiple nutritional stress (insufficient supply of K, P, Zn, Ca, Si) rather than by a high level of water-soluble iron under acid conditions (Ottow *et al.*, 1982).

Because of the various conditions causing iron toxicity and the high variability of the chemistry in flooded soils, screening for iron toxicity is best done in selected and adequately characterized field sites.

IRRI breeding lines tolerant to acid sulfate soils have been released especially in Vietnam (Table 4) while the most tolerant modern cultivars have been developed in Sri Lanka and Colombia (Table 5).

P deficiency

Phosphorus deficiency limits rice yields on Ultisols, Oxisols, acid sulfate soils, Andosols, and some Vertisols. P deficiency has been identified as the major nutrient deficiency on the 20 million ha cultivated to upland rice (Arraudeau, 1985). Because P is deficient in almost all problem soils some tolerance to P deficiency is essential for cultivars to be grown in these soils. The yield advantage of tolerant modern cultivars is often dramatic in P deficient soils (Table 6).

Zn deficiency

Zinc deficiency is the most common nutritional deficiency of wetland rice next only to N and P deficiency. It is now recognized as a limiting factor on calcareous soils, sodic soils, sandy soils, peat soils and regardless of pH on continuously wet soils (IRRI 1971, 1972).

Because IRRI's research farm has moderately Zn deficient soils all IRRI breeding lines were exposed to this selection pressure. Intensive screening and breeding has resulted in the high tolerance of all IRRI's late elite line (Fig. 2). The yield advantage of Zn-efficient cultivars in Zn-deficient soils is as dramatic as for P efficient cultivars in P-deficient soils (Table 6). On moderate Zn deficient soils grain yields of Zn efficient modern cultivars may even decrease if Zn is applied (Cayton *et al.*, 1985).

Table 6. Yield advantage of tolerant modern rices on problem soils in farmer's field in the Philippines, 1977–1988

Stress	Total number			Mean yield (t ha^{-1})		
	Tests	Sites	Rices	Farmer rices	Tolerant rices	Diff.
Salinity	50	20	120	1.4	3.4	2.0
Alkalinity	8	2	103	0.8	3.4	2.6
Iron toxicity	22	4	104	2.2	4.1	1.9
Peatiness	33	6	103	1.3	3.2	1.9
P deficiency	24	2	336	2.2	4.9	2.7
Zn deficiency	46	11	411	1.8	4.4	2.6
B toxicity	5	1	34	1.1	3.0	1.9
Fe deficiency	13	1	89	0.9	2.8	1.9
Al/Mn toxicity	10	3	44	1.2	3.0	1.8

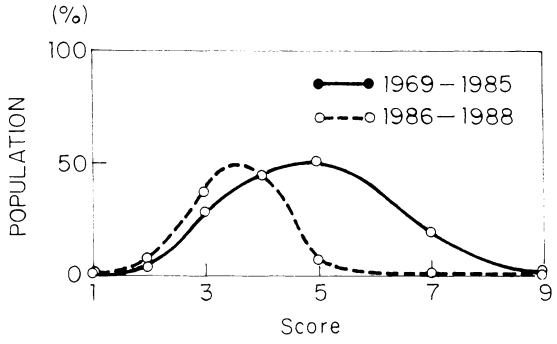

Fig. 2. Frequency distribution of scores for tolerance to Zn deficiency of IRRI's breeding lines.

Multiple soil stress tolerance

Rices that reveal some tolerance to salinity, P deficiency and Zn deficiency are often found to be tolerant to multiple soil stresses. Cultivars IR36 and IR42 (Table 4) show outstanding performance on various adverse soils. The multiple stress tolerance of IR36 has likely contributed to its cultivation on almost 11 million ha worldwide (IRRI 1982). That Zn, P and salinity tolerance seem to be crucial traits for multiple soil stress tolerance may be caused by linkages to other tolerance traits. Salinity tolerance scores are correlated with tolerance scores to alkalinity (0.15*) and peatiness (0.22*), P deficiency to alkalinity (0.17*) and Zn deficiency (0.19*) and Zn deficiency to Fe toxicity (0.34*).

Conclusions

Screening and breeding for adverse soil tolerance has successfully contributed to improved and more stable yields on fertile and infertile rice lands. There is possibility that millions of hectares of idle land can be brought under rice cultivation in Asia where land resources for agriculture become scarce.

Further knowledge on mechanism and inheritance of adverse soil tolerance are needed to enhance screening techniques and breeding success.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 533–538.
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PLSO PN 195

Genetic diversity for nutrient use efficiency in cultivars and exotic germplasm lines of alfalfa

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Key words: alfalfa, macro and micro nutrient uptake, nutrient culture system

Abstract

Alfalfa (*Medicago sativa* L.) is perhaps the most widely grown pasture legume in the world. Research was undertaken to evaluate the genetic diversity among selected cultivars and germplasm lines for growth, mineral uptake and mineral uptake efficiency ratios (ER). The ER is defined as milligrams of dry shoot weight produced per milligram of element in the shoot. Climatically controlled growth rooms were used to evaluate the performance of alfalfa in a nutrient culture system. Shoot and root dry weight of cultivars and germplasm lines gave a positive correlation with the uptake of various elements. The ER assisted in differentiating plants into efficient and inefficient utilizers of the absorbed nutrients. With few exceptions, the shoot and root dry weight gave positive relationships with ER for various elements. The cultivars and germplasm lines used in this study showed intraspecific genetic diversity in growth, nutrient uptake, and ER for the essential elements.

Introduction

Alfalfa (*Medicago sativa* L.) is probably the most widely grown pasture legume in the world. In North America alone, it is grown on 11 million ha (Bolten *et al.*, 1972). It is adapted to a wide range of climatic and soil conditions, but it is known to perform poorly in acidic infertile soils (Hanson and Barnes, 1973; Rhykerd and Overdahl, 1972). Poor performance of alfalfa in acid infertile soils is probably attributable to its inability to tolerate soil acidity and make use of the low levels of essential nutrients to support its growth.

Inter- and intraspecific variation for plant growth and mineral composition have been well documented and such differences are known to be under genetic control (Clark, 1984; Epstein and Jefferies, 1964; Gerloff and Gabelman, 1983; Kendall and Stringer, 1985; Vose, 1984). Genotypic and cultivar differences in shoot mineral concentrations have been reported for white clover (*Trifolium repens* L.) (Caradus, 1982; Davies *et al.*, 1968; Robinson, 1942; Snaydon, 1962), red clover (*Trifolium pratense* L.) (Baligar *et al.*, 1987; Hunt *et al.*, 1976; Seay and Henson, 1958), subclovers (*Trifolium subterraneum* L.) (Millikan, 1957), and alfalfa (Buss *et al.*, 1975; Hill and Jung, 1975). Differences in growth and elemental uptake among cultivars have been related to absorption, translocation, shoot demand, dry matter production potentials per unit of nutrient absorbed, and environmental interactions (Baligar *et al.*, 1987; Clark, 1984; Gerloff and Gabelman, 1983; Vose, 1984).

Identification of cultivars with greater tolerance to suboptimal conditions offers considerable promise for increasing the crop production potential of marginal lands throughout the world. Subjecting diversified cultivars and germplasm lines to a uniform nutrient supply will assist in separating the plant entries into superior and inferior nutrient utilizers. Superior entries could be useful to breeders as gene donors and to geneticists as a source of well defined physiological traits to be used in inheritance studies related to plant nutrition.

The objective of the present experiment was to evaluate cultivars and exotic germplasm lines of alfalfa for genetic diversity in growth, mineral uptake and mineral efficiency ratios in a nutrient culture system.

Materials and methods

Cultivars, lines and germplasms

Experiment 1 involved 17 cultivars and 3 lines (Table 1). The lines B-24, B13-Al-4, and B13-AL4(F1) have been shown to have some degree of aluminum tolerance. The cultivars used are well adapted to various alfalfa growing regions of North America. Experiment 2 included 40 germplasm lines obtained from the USDA-ARS Regional Plant Introduction Station in Ames, Iowa. These germplasm lines have been obtained from important alfalfa growing regions of the world.

Experimental conditions

Both experiments were conducted in climatically controlled growth chambers where 14 h of 530 μmol s^{-1} m^{-2} light intensity was imposed. The day temperature was 28°C and relative humidity was 60%. In the dark period, temperature and relative humidity were 25°C and 80%, respectively. Four 6-day-old seedlings were placed in 6.5 cm diameter styrofoam rings fitted to bottomless plastic containers. Fifteen of these plastic containers were suspended over 14 L polyethylene tubs fitted with high density styrofoam tops. A complete randomized design with three replications was used. Modified one-fifth strength Steinberg solution with an initial pH of 4.5 was used. Within a week, solution pH was greater than 5.5, and no further adjustment in pH was made during the growth. The nutrient solution had the following composition: 3.7 m*M* NO_3-N, 0.3 m*M* NH_4-N, 1.3 m*M* Ca, 0.3 m*M* Mg, 0.7 m*M* K, 97 μM P, 115 μM S, 6.6 μM B, 0.6 μM Zn, 0.16 μM Cu, 0.10 μM Mo, 2.36 μM Mn and 17.9 μM Fe. The Fe

Table 1. Alfalfa cultivar and germplasm lines and their origin and seed source

Cultivar/lines				Germplasm lines[b]					
No.	Name	Origin	Seed[a] Source	No.	PI[c] No.	Origin	No.	PI No.	Origin
1	Agate	USDA Minnesota AES	1	21	190258	Algeria	41	188795	Sweden
2	Apalachee	USDA & North Carolina AES	2	22	190259	Algeria	42	179368	Syria
3	Apollo	North Amer. Plant Breeder	3	23	145202	Arabia	43	181889	Syria
4	Arc	USDA	3	24	183261	Arabia	44	181915	Syria
5	Atlantic	New Jersey AES	3	25	162787	Argentina	45	170536	Turkey
6	B-24	USDA-BARC	2	26	185858	Argentina	46	167263	Turkey
7	B13-AL-4	USDA-BARC	2	27	286361	Australia	47	171720	Turkey
8	B13-AL-4(F1)	USDA-BARC	2	28	292773	Australia	48	175788	Turkey
9	Baker	USDA & Nebraska AES	1	29	259520	Bulgaria	49	178981	Turkey
10	Cimarron	Great Plains Res. Co.	4	30	206104	France	50	182242	Turkey
11	DuPuits	Tourneur Freres	2	31	179946	India	51	179371	Turkey
12	Grimm	Minnesota Ecotype	2	32	179947	India	52	180854	Turkey
13	Narragansett	Rhode Island AES	2	33	183328	India	53	182238	Turkey
14	Ramsey	USDA-Minnesota AES	1	34	141462	Iran	54	182239	Turkey
15	Ranger	USDA-Nebraska AES	1	35	141458	Iran	55	187003	USA-Iowa
16	Saranac	Cornell Univ., NY	3	36	286365	Israel	56	86696	USSR
17	Shenandoah	Great Plains Res. Co.	4	37	189393	New Zealand	57	162457	Uruguay
18	Spredor II	Northrup King Co.	3	38	247790	Peru	58	162459	Uruguay
19	Team	USDA-NC, MD, VA AES	1	39	172189	Peru	59	162458	Uruguay
20	Vernal	USDA-WS and UT AES	1	40	172190	Peru	60	193291	Yugoslavia

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[b] Seeds received from Dr R L Clark, Regional Plant Introduction Station, Ames, Iowa.

[c] PI = Plant Introduction.

was added as equal amounts of $FeSO_4$ and FeDTPA. Solutions were renewed when crops were 23-days old and at this stage, P levels of the solutions were doubled. During growth, solutions were aerated continuously. Plants were harvested after 35 days of growth. At harvest, shoots and roots were separated, washed several times in deionized water, and blotted dry. Plants were oven dried at 65°C for 3 days and dry weights for shoots and roots were recorded. In addition, shoot height was measured. Shoot samples were ground to pass a 0.55 mm mesh sieve and wet digested in a HNO_3:$HClO_4$ (4:1) mixture. Elemental determinations were made by inductively coupled plasma emission spectroscopy (ICP). The nutrient uptake efficiency ratio (ER) for various elements was calculated as follows (Gerloff and Gabelman, 1983): ER = milligrams of dry shoot weight/milligrams of element in shoot.

Results and discussion

Growth of shoots and roots

On an average, alfalfa germplasm lines yielded 13% higher dry weight of shoots and 43% higher dry weights of roots than the cultivars (Table 2). Generally, germplasm lines were taller than cultivars. Shoot/root ratios of cultivars were higher than those of germplasm lines indicating a lower allocation of dry matter to the root system in the cultivars. Among cultivars, Cimarron, Spredor II, Saranac and B13-AL-4 had the highest shoot dry wt. and Spredor II and Saranac had the highest root dry wt. Agate and Grimm had the lowest shoot and root dry weight (Data not shown). Among germplasm lines, the highest shoot and root dry wt. were recorded in PI-183328 and PI-190259, respectively. The line PI-286361 produced the lowest dry weight both in shoots and roots (Data not shown).

Nutrient concentrations

The productivity of a plant can be influenced by its elemental composition and further, elemental composition of a plant has a profound effect on animals that consume it. Table 3 gives the mean of various shoot elemental concentrations in cultivars

Table 2. Mean and range of plant growth parameters of cultivars and germplasm lines

Plant parameters	Cultivar		Germplasm lines	
	Mean	Range	Mean	Range
Shoot dry wt (g/plant)	0.61	0.11–0.99	0.70	0.22–1.66
Root dry wt (g/plant)	0.14	0.03–0.28	0.20	0.05–0.44
Shoot height (cm)	53.00	25.00–70.00	60.00	37.00–97.00
Shoot/root	4.49	2.22–7.77	3.56	1.85–5.78

Table 3. Average values for concentrations (C), uptake (U), and uptake efficiency ratios (ER) for various elements in cultivars and germplasm lines[a]

Element	Cultivars			Germplasm lines		
	C	U	ER[b]	C	U	ER[b]
N	23.5	14.3	44	22.7	16.0	45
P	1.9	1.2	532	1.8	1.2	584
S	1.5	0.9	684	1.5	1.0	687
K	16.3	9.8	63	15.1	10.5	68
Ca	13.8	8.0	82	15.3	10.8	69
Mg	1.2	0.7	897	1.4	1.0	750
Mn	38.9	23.5	28	41.3	28.9	26
Fe	130.7	75.9	10	151.7	100.7	9
Cu	19.6	11.7	54	15.1	10.4	69
Zn	37.2	22.1	28	29.8	20.4	36

[a] C = $mg\,g^{-1}$ for N, P, S, K, Ca and Mg; $\mu g\,g^{-1}$ for Mn, Fe, Cu and Zn. U = $mg\,plant^{-1}$ for N, P, S, K, Ca and Mg; $\mu g\,plant^{-1}$ for Mn, Fe, Cu and Zn.

[b] ER = See Equation 1. Multiply by 10^3 for Mn, Fe, Cu and Zn.

and germplasm lines. Concentrations of Ca, Mg, Mn and Fe, tended to be higher in the shoots of germplasm lines than in those of cultivars. Concentrations of P and S were similar in the shoots of germplasm lines and cultivars. Higher elemental concentrations in cultivars could be attributable to lower dry matter accumulation in the shoots (Table 2). Concentrations of various elements were different among various cultivars and germplasm lines. Variations in elemental concentration among alfalfa genotypes and cultivars have been reported previously (Buss *et al.*, 1975; Hill and Jung, 1975). Yield of alfalfa is reported to be negatively correlated with various mineral concentrations (Hill and Barnes, 1977; Hill and Jung, 1975). In the current study, correlations between shoot dry weight and elemental concentrations were negative; however, correlations were significant only with Ca, Zn, N and Cu (Data not shown).

Nutrient uptake (U)

With the exception of Cu and Zn, the mean uptake for other nutrients in cultivars was lower than in germplasm lines (Table 3). With the exception of Fe uptake by cultivars, the shoot and root dry matter gave a highly significant positive correlation with elemental uptake (Table 5). Plant demand for any given nutrient is a function of its internal ionic concentrations and its rate of growth (Pitman, 1972; White, 1973; Williams, 1948).

The most and least efficient cultivars and germplasm lines for uptake of various nutrients are given in Table 4. The cultivars Narragansett, Spredor II, Saranac and Apollo showed the highest efficiency in uptake of P, Ca, Mg and Zn, respectively. The line B13-AL-4 gave the highest efficiency in accumulation of N, S and Mn. The cultivar Agate was the most inefficient in uptake of N, P, K, Mg, Mn, Fe and Cu. This may be a reflection of Agate's low dry shoot wt. The efficient nutrient accumulators showed higher shoot dry wt.

Line PI-183328 from India was very efficient in taking up N, P, K, Fe and Cu. This germplasm line also had highest shoot dry wt. The Yugoslavian line PI-193291 was very efficient in uptake of S and

Table 4. The uptake (U) and uptake efficiency ratios (ER) for different elements of the most and least efficient alfalfa cultivars and germplasm lines[a]

Element	Uptake				ER			
	Most efficient		Least efficient		Most efficient		Least efficient	
	No	U	No	U	No	ER	NO	ER
Cultivars								
N	7	17.8	1	8.9	5	54	12	34
P	13	1.5	1	0.5	5	629	13	403
S	7	1.2	3	0.4	1	909	9	527
K	15	12.5	1	4.2	5	78	12	48
Ca	18	10.6	3	4.8	17	102	1	58
Mg	16	0.9	1	0.3	1	1091	11	734
Mn	7	31.4	1	1.0	17	44	13	20
Fe	10	170.3	1	21.3	5	19	14	4
Cu	4	15.9	1	7.3	17	71	12	43
Zn	3	28.5	12	13.1	19	35	1	18
Germplasm lines								
N	33	30.5	27	8.8	44	61	27	37
P	33	2.3	27	0.6	40	741	29	474
S	60	1.8	27	0.6	34	1161	52	552
K	33	20.7	27	6.1	57	84	29	54
Ca	22	19.5	27	4.7	34	141	23	49
Mg	60	1.7	27	0.4	34	1128	35	584
Mn	22	52.3	27	15.3	34	47	51	20
Fe	33	245.0	52	37.4	59	13	35	43
Cu	33	18.5	52	6.0	34	106	57	41
Zn	22	40.3	52	12.7	47	44	27	20

[a] Refer to Table 1 for identification of cultivars and germplasm lines and refer to Table 3 for units of measurements for U and ER.

Table 5. Correlation coefficient (r) values for growth, U, and ER for different nutrients against shoot and root growth of cultivars and germplasm lines[a]

	Cultivar		Germplasm lines	
	Shoot dry wt	Root dry wt	Shoot dry wt	Root dry wt
Shoot dry wt	1.00	0.62**	1.00	0.84**
Shoot height	0.51**	0.32*	0.57**	0.45**
Root dry wt	0.62**	1.00	0.84**	1.00
U-N	0.79**	0.45**	0.92**	0.75**
U-P	0.84**	0.42**	0.92**	0.75**
U-S	0.84**	0.42**	0.90**	0.75**
U-K	0.83**	0.41**	0.91**	0.69**
U-Ca	0.70**	0.58**	0.90**	0.78**
U-Mg	0.77**	0.67**	0.91**	0.76**
U-Mn	0.74**	0.53**	0.87**	0.73**
U-Fe	0.22NS	0.13NS	0.42**	0.31**
U-Cu	0.68**	0.44**	0.82**	0.70**
U-Zn	0.80**	0.44**	0.81**	0.68**
E-N	0.30*	0.13NS	0.09NS	0.09NS
E-P	0.19NS	0.28*	0.09NS	0.15NS
E-S	−0.03NS	0.05NS	0.15NS	0.18*
E-K	0.17NS	0.22NS	0.16NS	0.32**
E-Ca	0.12NS	−0.18NS	−0.07NS	−0.06NS
E-Mg	−0.01NS	−0.30*	0.06NS	0.06NS
E-Mn	−0.02NS	−0.14NS	0.06NS	0.10NS
E-Fe	0.24NS	0.16NS	0.18*	0.21*
E-Cu	0.25NS	0.11NS	0.18*	0.19*
E-Zn	0.30*	0.27*	0.10NS	0.08NS

[a] *, ** Significant at 0.05 and 0.01 probability levels, respectively and NS = Not significant.

Mg, whereas line PI-190259 from Algeria was very efficient in taking up Ca, Mn and Zn. The Australian line PI-286361 was most inefficient in accumulation of N, P, S, K, Ca, Mg and Mn, and also had the lowest shoot and root dry wt. The lowest accumulations of Fe, Cu and Zn were observed with PI-180854 from Turkey.

Nutrient uptake efficiency ratios (ER)

The calculated ER is a valuable parameter in differentiating plants into efficient and inefficient utilizers of the absorbed nutrients. ER values for Ca, Mg, Mn and Fe tended to be lower in germplasm lines than in cultivars (Table 3). With the exception of ER values for S, Mg and Mn, the ER for other elements gave a positive correlation with shoot dry weight of cultivars; however, correlations were significant only for N and Zn (Table 5). In germplasm lines, with the exception of Ca, shoot and root weight were positively correlated with ER values.

The cultivars and germplasm lines behaved differently for nutrient uptake and ER (Table 4). Any one cultivar or germplasm line is neither most nor least efficient in utilization of all absorbed elements. The cultivar Atlantic was very efficient in utilizing absorbed N, P, K and Fe, whereas, Agate was efficient in utilizing absorbed S and Mg. Shenandoah was efficient in utilizing absorbed Ca, Mn and Cu. The cultivars Narragansett, Agate and DuPuits were inefficient in utilization of P and Mn, Ca and Zn, and Mg, respectively.

Line PI-141462 from Iran gave high ER values for S, Ca, Mg, Mn and Cu. A high ER for P was observed in PI-172190 from Peru. Lines PI-259520, PI-145202, and PI-141458 gave the lowest ER for P, Ca and Mg respectively. The germplasm line PI-286361 from Australia recorded the lowest ER for N and Zn. This line recorded the lowest uptake

for N, P, S, K, Ca, Mg and Mn and also had the lowest shoot wt.

The cultivars and germplasm lines used in this study showed intraspecific diversity in nutrient uptake efficiency. The intraspecific variation in nutrient uptake and utilization in various other crops is well documented (Baligar *et al.*, 1987; Clark, 1984; Epstein and Jefferies, 1964; Gerloff and Gabelman, 1983; Kendall and Stringer, 1985; Vose, 1984). From the present study it could be concluded that efficient nutrient utilizers could be used in breeding programs to evolve superior cultivars for any given elemental stress ecosystem.

Acknowledgements

We thank Drs J L Ahlrichs, T A Campbell, R N Singh and C D Foy for their peer review of the manuscript. The technical assistance of M D Smedley is greatly appreciated.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 539–545.
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PLSO PN 196

Breeding for low level acid soil tolerance as a component of overall acid soil field tolerance in sorghum

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Key words: acid soil pH, alachlor, aluminum, genetics, gibberellin, glutathione, herbicide tolerance, manganese, metolachlor, *Sorghum bicolor* (L.) Moench, toxicity

Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is quite sensitive to acid soil stress conditions. A two-phase sequential breeding program has resulted in development of low level acid soil tolerance = suspected Mn toxicity tolerance (Phase I) and Al toxicity tolerance (Phase II) under field conditions. Separation of specific tolerance mechanism(s) has revealed some interesting speculations. Sorghum plants are thought to undergo three very distinct growth responses (impact, pre-flowering, post-flowering) to each toxicity condition. Initial investigations into the suspected Mn toxicity × impact response phase (germination to panicle initiation = ~40 days) have resulted in a possible relationship between low level acid soil tolerance and metolachlor/alachlor resistance. This association appears to involve enzyme-mediated glutathione conjugation/GA activity and the Mg-Mn balance in the plant. Field evaluations have indicated a possible 'preconditioning' of genotypes during low level acid soil tolerance development before the more severe Al toxicity tolerance developmental phase can be implemented.

Abbreviations. metolachlor = [2-chloro-*N*-(2-ethyl-6-methyl-phenyl)-*N*-(methoxy-1-methylethyl)-acetamide] alachlor = [2-chloro-N-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide]

Introduction

Sorghum cultivars differ in their response to acid soil stress and its associated Mn toxicity conditions (Brown and Jones, 1977). The critical Mn concentration (adequacy) in sorghum [*Sorghum bicolor* (L.) Moench] leaf tissue of juvenile plants has been established as 10 to 60 $\mu g\,g^{-1}$ (Ohki, 1975). In nutrient solutions, high Mn concentrations of 2000–3000 $\mu g\,g^{-1}$ in sorghum leaves did not inhibit shoot growth, but root Mn concentrations exceeding 1000 $\mu g\,g^{-1}$ inhibited root growth (Galvez *et al.*, 1987). Interactions between Mn and P, Fe, Zn, Mo, Si, B, Cu and Ca are well documented (Foy, 1984; Foy and Fleming, 1978; Kuo and Mikkelsen, 1981). Excellent reviews dealing with biochemical and physiological processes influenced by Mn toxicity are available (Clark, 1982ab; Foy, 1983; 1984). A sequence of events for plant response to Mn toxicity has been proposed (Sirkar and Amin, 1974). This sequence involves 1) accumulation of Mn in young leaf tissue, 2) escalation of respiration, 3) increase in polyphenol oxidase activity, 4) appearance of initial Mn toxic symptoms in young leaves, 5) evolution of ethylene and stimulation of peroxidase, 6) progression to extremely severe symptoms, 7) depression of respiratory terminal oxidases and depression of respiration, 8) abscission of growing tips and, 9) proliferation of tissues.

Differential tolerance

Significant inter- and intraspecies differences in plant response to excess Mn have been found (Foy,

Table 1. Documented differential tolerance responses to excess Mn within selected species

Type of tolerance	Crop	Reference
Tolerance not related to Mn concentration in plant, but to form of Mn(Mn^{4+} or Mn^{2+}) internally	*Glycine max* L. Merr	Brown and Devine, 1980
Genotypes have different levels of internal tolerance to excess Mn	*Gossypium hirsutum* L.	Foy *et al.*, 1981
Differential internal tolerance to Mn toxicity and not decreased Mn uptake	X Triticosecale	Mugwira *et al.*, 1981
	Triticum aestivum L. em Thell	Neenan, 1960
Greater ability to tolerate high level of Mn accumulation in leaves	*Phaseolus vulgaris* L.	Kohno and Foy, 1983
Tolerance not related to reduced Mn uptake	*Oryza sativa* L.	Nelson, 1983
Mn-hyperaccumulator plant, malate functions as transport vehicle through cytoplasm and oxalate as terminal acceptor in vacuole (*i.e.* Mn chelation by oxalate is detoxification mechanism)	*Acanthoparax sciadophylloides*	Memon and Yatazawa, 1984

1984). Additional studies have dealt with Mn toxicities (Dessureaux and Ouellete, 1958; Heenan and Carter, 1976; Neenan, 1960; Ouellette and Dessureaux, 1958; Sanchez and Salinas, 1981). Plant tolerance to Mn has been associated with 1) oxidation within the root or root rhizosphere, 2) differential absorption and translocation rate, 3) entrapment or compartmentalization in non-metabolic centers, 4) high internal tolerance, 5) complexation or detoxification, 6) rate of uptake and distribution of Si and Fe (Foy, 1984). Considerable research has implicated differential uptake/transport and differential internal tolerances as predominant mechanisms (Andrew, 1976; Edwards and Asher, 1982; Foy, 1983; 1984). A summary of tolerance responses within six plant species is found in Table 1. Internal tolerance differences have been implicated for bush bean, soybean, cotton, and triticale.

The exact internal tolerance mechanism is unknown. Memon and Yatazawa (1984) determined that a detoxification mechanism involving oxalate was functioning in a Mn-accumulator plant.

Genetic control of excess Mn

Several reviews are available on breeding methodology and genetic potential for solving Mn toxicities in various species (Clark and Brown, 1980; Devine, 1976; 1982; Foy, 1983; Reid, 1976; Saric, 1981; 1983; and Thung *et al.*, 1987). Inheritance studies involving plant adapatation to Mn toxicities have been reviewed by Foy *et al.* (1988). Data on four species listed in Table 2 generally agreed that more than one gene was involved in the tolerance mechanism, but data on mode of inheritance ranged from strictly additive effects (alfalfa, maize) to partial dominance (soybean) to complete dominance (lettuce). Sorghum tolerance to Mn toxicity under acid soil field conditions has not been documented, but preliminary results from the acid soil stress-oriented sorghum breeding program in Georgia indicates a multigenic situation with some form of dominance involved (Duncan, 1988). A recent evaluation of selected exotic accessions revealed a surprisingly high level of tolerance to low level acid soil tolerance or suspected Mn toxicity tolerance in field plots (Table 3). One-fourth of

Table 2. Genetic control of Mn toxicity tolerance in various crop species

Crop	Scientific name	Gene control/inheritance	Reference
Alfalfa	*Medicago sativa* L.	Polygenic/additive, no dominance	Dessureaux, 1959
Lettuce	*Latuca sativa* L. × *L. serriola*	1–4 genes/dominance	Eenink and Garretsen, 1977
Soybean	*Glycine max.* L. Merr.	Polygenic/cytoplasmic effect	Brown and Devine, 1980
		Polygenic/partial dominance	Heenan *et al.*, 1981
Maize	*Zea mays* L.	2–3 genes/additive	Gorsline *et al.*, 1968

Table 3. Evaluation of selected exotic sorghums for tolerance to suspected Mn toxicity field stress conditions

Country	Total number evaluated	Visual rating scale[c]			
		1.0–1.5 Tolerant	2.0–2.5	3.0–3.5	4.0–5.0 Susceptible
			Intermediate		
South Africa	353	86 (24)[b]	218 (62)	43 (12)	6 (2)
Zimbabwe	358	11 (3)	123 (34)	214 (60)	10 (3)
Uganda	140	5 (4)	44 (31)	84 (60)	7 (5)
French Equitorial Africa	5	—	4	1	—
Tanzania	1	1	—	—	—
Subtotal	857	103 (12)	389 (45)	342 (40)	23 (3)
Converted exotics[a]	240	55 (23)	71 (30)	37 (15)	77 (32)

[a] Diverse group of exotics converted to short, early, photoperiod insensitive lines with original exotic cytoplasm (Stephens *et al.*, 1967). Conversion was conducted primarily on plants grown on alkaline soils in Texas and on soils above pH 6.0 in Puerto Rico.
[b] Numbers in parentheses represent % of total.
[c] Duncan, R R 1981 Visual field rating scheme for plants grown on acid soils. Sorghum Newsl 24, 69 and Duncan *et al.*, 1983c.

the exotics from South Africa had a high level of tolerance, while less than 5% of the accessions from Zimbabwe and Uganda had a high level of tolerance. Among the 850 lines evaluated, 85% had an intermediate level of tolerance and only 3% were susceptible. Consequently, sorghum germplasm sources are available which have an intermediate to high level of possible Mn toxicity tolerance (or low level acid soil tolerance).

The objective of this paper is to describe a sorghum breeding program in the southeastern USA which involves development of genotypes sequentially for two levels of acid soil tolerance *i.e.* suspected Mn and Al toxicity tolerances. The em-

Table 4. Soil stress environments for the acid soil tolerance sorghum breeding program

Location	Soil type[f]	Soil pH		Ml extr.[b]		KCl[c] Al	NH_4OAc[d]			Al[e] sat.
		H_2O	KCl	Mn	P		Ca	Mg	K	
				μg/g			$cmol_c\,kg^{-1}$			%
Blairsville	Dyke cl	4.7	4.1	62[a]	19	0.80	2.23	0.32	0.61	20
		4.1	3.8	62	14	2.03	0.25	0.06	0.49	71
	Congaree sl	4.8	4.1	52[a]	9	1.57	1.41	0.18	0.52	42
		4.1	3.8	42	4	1.96	0.49	0.11	0.48	64
Rome	Cedarbluff sl	4.8	4.0	36[a]	22	0.63	1.51	0.30	0.30	23
		4.3	3.7	37	26	1.16	0.73	0.17	0.32	48
Griffin	Cecil scl	4.5	4.0	30[a]	6	0.59	1.24	0.26	0.21	25
		4.1	3.8	26	20	1.47	0.46	0.09	0.24	63
	Pacolet scl	4.6	3.8	6	3	1.67	1.05	0.50	0.27	48
		4.4	3.8	10	3	2.02	0.97	0.25	0.34	56
		4.1	3.7	6	10	1.74	0.15	0.05	0.15	83
	Appling csl	4.7	4.2	40[a]	8	0.38	1.24	0.17	0.13	19
		4.1	4.0	29	9	0.79	0.76	0.12	0.08	45

[a] Used for low level acid soil tolerance or suspected Mn toxicity screening (Phase 1).
[b] Extracted using Mehlich 1 (Southern Coop. Series Bull. 102, 1965).
[c] Extracted with 1 *N* KCl (Barnhisel and Bertsch, 1982).
[d] Exchangeable cations extracted with 1 *M* NH_4OAc, pH 7 (Thomas, 1982).

[e] Percent Al saturation of the CEC $= \dfrac{Al(cmol_c\,kg^{-1})}{Al + Ca + Mg + K + Na(cmol_c\,kg^{-1})} \times 100$

[f] Dyke = Typic Rhodudults; Congaree = Fluventic Dystrochrepts; Cedarbluff = Fragiaquic Paleudults; Cecil, Pacolet, Appling = Typic Hapludults.

Table 5. Relationship of Mn tolerance and metolachlor resistance in selected sorghum genotypes[a]

Genotype	Overall acid soil field stress reaction	Mn tolerance	Metolachlor resistance	Metolachlor degradation
SC283	very tolerant	high	low	low
SC599	intermediate	low	high	high
GP 140	intermediate	high	low	intermediate
TAM428	susceptible	intermediate	high	intermediate

[a] From Wilkinson *et al.* (1988).

phasis of this paper will be on the low level acid soil (or suspected Mn toxicity) tolerance.

Methods

All screening and evaluations were conducted in Georgia. Previous publications have described the soil stress environments (Duncan, 1981a; 1982; 1983abc; Duncan *et al.*, 1980). A summary of the 1987 soil data is included in Table 4. Soil pH was determined on 1:2 soil to solutions suspensions for water and 1 *N* KCl. Manganese and P were extracted using the Mehlich 1 procedure (Southern Coop. Bull. 289, 1983) and the P determined colorimetrically. Exchangeable Al was extracted using 1 *N* KCl (Barnhisel and Bertsch, 1982) and exchangeable bases were extracted using 1 *M* NH_4OAc after the method of Thomas (1982). Exchangeable Al, Ca and Mg and the extractable Mn were analyzed using atomic absorption and exchangeable K and Na by emission spectroscopy. The percent Al saturation was calculated by dividing the exchangeable Al by the sum of the exchangeable Al, Ca, Mg, K, and Na and multiplying by 100.

Generally, the soils with pH (water extraction) of 4.5 to 4.8 are used for low level acid soil tolerance or suspected Mn toxicity screening. In general, these ultisols are characterized by (1) low organic matter (less than 1.5%) (2) coarse texture and poorly developed structure (3) moderate to strong acidity (4) low base status and high leachability (5) high weatherability with predominately 1:1 non-expanding clays (6) low P availability, high P fixing capacity, and high Fe- and Al-oxide contents (7) high Mn availability at moderate (water pH 4.5–5.0) acidity levels (8) low water infiltration rates and (9) high Al availability at soil pHs below 4.5.

Results

A schematic of the overall dual-phase acid soil tolerance developmental program for sorghum is presented in Figure 1 (Duncan, 1988).

Phase 1 = Low level acid soil tolerance or suspected Mn toxicity tolerance screening

Selection of tolerant genotypes is accomplished at a soil pH range of 4.5 to 4.8 (water extraction) on six soils at three locations. Aluminum saturation values range from 19–48% (Table 4). The germplasm base is generally broad enough to provide sufficient genotypes for proper segregation patterns. By-passing Phase 1 or moving too rapidly during Phase 1 development can eliminate potentially favorable genotypes and narrow the germplasm base to the point of detrimentally affecting the breeding program. Manganese toxicity has been established as a major problem for sorghum during Phase 1 screening (Wilkinson *et al.*, 1988); however, low level Al-toxicity cannot be ruled out. Experimental hybrids are initially evaluated on this level of stress at 1–2 locations as well as at a pH greater than 6.0 at Griffin. Subsequent segregating generations are subjected to a minimum of two locations per year, depending on seed availability. Surviving pedigrees from one location and/or soil type are grown at the remaining two locations the following year. Consequently, during Phase 1 development (average of 5 to 8 generations), segregating genotypes are subjected to acid soil stress conditions at all 3 locations and involving 6 different soils. During the F_2 to F_4 generations, family selection is exercised due to the large number of pedigrees in the program and the possible 'escapes' evolving from environmental con-

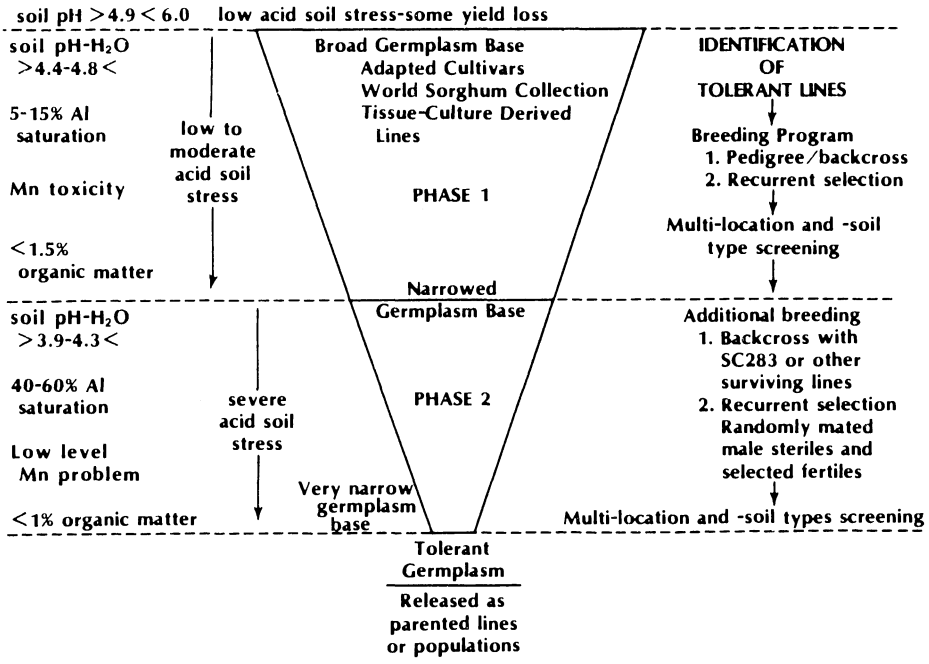

Fig. 1. Schematic of acid soil constraints, narrowing of germplasm base during development and breeding approaches during the dual-phase program.

ditions other than acid soil stress (*i.e.* excess rainfall). Selection indices for the conventional (pedigree-backcross) approach generally range from 5 to 15% depending on the pedigrees involved and the environmental conditions at each location. Recurrent selection techniques involving random-mating populations have been the most rapid method for developing tolerant pedigrees (Duncan, 1981b; 1988). Randomly-mated male steriles as well as the best 5–10% male fertile panicles are selected for recombination each generation. Two generations per cycle and a minimum of 3 cycles of random mating are used to develop sufficient levels of suspected Mn toxicity tolerance in the populations. An acid tropical soil (pH 4.0–4.5) is used in the Puerto Rico winter nursery program to enhance the breeding efforts (Al toxicity is suspected as the major growth inhibitor). General adaptability, agronomic desirability, and disease resistance characteristics can be incorporated during Phase 1 development.

Phase 2 = Al toxicity tolerance screening

This phase of the breeding program will not be discussed here. Refer to Duncan (1988) for a further explanation. However, tolerance to excess Al was not correlated with tolerance to excess Mn in upland rice (Nelson, 1983). Al saturation values for the 6 soils range from 45–83%, with most severe stress levels in the 48–64% range (Table 4).

Low level acid soil tolerant germplasm releases

A random-mating population (Duncan, 1981b) and 3 male fertility restorer lines (Duncan, 1984) have been released. Additional germplasm emerging from Phase 1 development are automatically moved into the Phase 2 program. Apparently, some form of 'preconditioning' from Phase 1 development is necessary before genotypes can effectively withstand Phase 2 acid soil stress (Al toxicity). Whether this preconditioning involves solely tolerance mechanism(s) to excess Mn, low-level Al toxicity, to the gradual improvement of the root system, or all of the above is unknown.

Relationship between Mn tolerance and metolachlor resistance

Data in Table 5 and a companion paper in the

symposium (Wilkinson *et al.*, 1988) support an association between low level Al tolerance and/or suspected Mn toxicity tolerance and metolachlor herbicide resistance. Mn toxicity has been enzymatically linked to accelerated polyphenol oxidase activity in cotton (Sirkar and Amini, 1974). Elevated peroxidases and reduced catalase, ascorbic acid oxidase, glutathione oxidase, and cytochrome C oxidase activities were detected. Morgan *et al.* (1966) found a direct relationship between the degree of enzyme response and severity of visible Mn toxicity symptoms in cotton. They reported that Mn toxicity was associated with an increase in IAA-oxidase activity and a decrease in IAA-oxidase inhibitor activity. Vlamis and Williams (1967) found that Mn toxicity was expressed as an auxin deficiency in Gramineae, caused by high IAA oxidase activity. Anderson and Evans (1956) reported an increased synthesis of both isocitric dehydrogenase and a 'malic' enzyme in snapbean (*Phaseolus vulgaris* L.) leaves with elevated Mn levels. Mn deficiency had no effect on these enzymes.

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N. El Bassam et al. (Eds.), Genetic aspects of plant mineral nutrition, 547–552.
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PLSO PN 197

A programme to breed a cultivar of *Trifolium repens* L. for more efficient use of phosphate

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Key words: breeding, cultivars, phosphate, phosphate efficiency, white clover

Abstract

A programme designed to breed a cultivar of *Trifolium repens* L. (white clover) with a more efficient phosphate nutrition is in progress. This effort seeks to reduce the high requirement of white clover—grass pastures for phosphate fertiliser. The first stage has demonstrated substantial variation between cultivars of white clover in their response to added phosphate. This result is encouraging but it cannot be assumed that this variation will be useful in breeding a cultivar.

We are currently seeking to test the repeatability of the differences obtained previously and to identify promising genotypes from the cultivars which performed best. Subsequent phases of the programme will measure heritability of differences in phosphate nutrition, assess performance of differing genotypes in the field and attempt to identify physiological and morphological traits which contribute to improved performance.

With genotypes with heritable differences in their phosphate nutrition and information on their performance in the field and the characteristics which contribute to this, we should be in a position to proceed with the selection and breeding required to produce a cultivar.

Introduction

New Zealand relies heavily on phosphate fertilisers to maintain its agricultural production from pastures based predominantly on *Trifolium repens* L. (white clover)—grass swards. This system has lower fertiliser costs than an all grass system in which nitrogen is supplied as fertiliser (Ball and Field, 1985). Furthermore the pollution associated with it is considerably less (Ball and Field, 1985). However white clover competes ineffectively with grasses for phosphate and this nutrient has to be applied regularly to maintain productivity. This is the principal reason for New Zealand's position among the world's highest per capita users of phosphate. A cultivar of white clover requiring lower inputs of phosphate fertiliser would be of considerable value in maintaining the profitability of our pastoral farming.

A number of studies indicate genetic variation in the response to phosphate by white clover (Caradus and Dunlop, 1978; Caradus *et al.*, 1980; Mackay *et al.*, 1988; Snaydon and Bradshaw, 1962). This suggests that it would be worthwhile attempting to breed a phosphate efficient cultivar and we have embarked on a major programme to do this.

With present understanding we are reluctant to give more than a qualitative definition of a phosphate efficient white clover—a plant that requires less phosphate to sustain the same production as present cultivars or that produces more herbage with the same amount of phosphate.

This programme involves eight scientists with expertise in agronomy, soil science, plant breeding,

ecophysiology, plant physiology, biophysics and statistics. At peak periods technical assistance from up to ten further people is required. We estimate seven years will be required to complete this programme. With this investment we are anxious to maximise our chance of succeeding. There are few precedents, if any, of direct relevance to guide us. Therefore we hope that this paper will elicit advice which will facilitate the programme.

Significant features of white clover

Several aspects of white clover and its growth in mixed swards are important in considering the characteristics needed for a phosphate efficient cultivar.

White clover has a highly plastic form which is greatly influenced by the conditions in which it is grown (Brougham *et al.*, 1978). Plants grown in pots tend to be more erect and remain integrated whereas in pasture they are prostrate and based on fragments of stolon that have become detached from the parent plant (Chapman, 1983; Erith, 1924; Kershaw, 1959). Consequently, experimental plants are often morphologically different from field material which introduces doubt when extrapolating experimental data to field situations.

White clover is a strongly outbreeding species which exhibits large diversity even among the individuals of a given cultivar (Williams, 1987). This variation requires that genetic differences have to be studied using cloned ramets rather than seedlings thus increasing the labour required.

Agronomic considerations

It is expected that a sown pasture will remain productive for many years. Consequently the desired cultivar will have to be persistent as well as productive.

The plant will be grown in a sward with other species and therefore it will have to contend with interspecific competition. White clover is frequently at a disadvantage in competing for mineral nutrients (Dunlop and Hart, 1987; Harris, 1987; Jackman and Mouat, 1972).

Harvesting the plant involves repeated defoliations by grazing animals. Defoliation affects morphology (Thomas, 1987), root growth, nitrogen fixation and photosynthesis (Hart, 1987). This means that the performance of a potential cultivar has to be assessed over many seasons.

Because of animal returns of dung and urine there will be a mosaic of different levels of nutrient availability throughout the pasture. In other species this has important effects on growth patterns and foraging for nutrients (Harper, 1983; Sloade and Hutchings, 1987).

These aspects of pasture and its management have to be considered in developing a concept of a practical cultivar.

Philosophical basis of the programme

We have previously investigated a range of aspects of the phosphorus nutrition of white clover including its root morphology (Caradus, 1977; 1979; 1981a, b), relationship with myhcorrhizae (Crush and Caradus, 1980); root anatomy (Hay *et al.*, 1986a); absorption into the root (Bowling and Dunlop, 1978; Dunlop and Bowling, 1978; Dunlop *et al.*, 1984; Hay *et al.*, 1986a, b) and its relationship with proton extrusion (Dunlop *et al.*, 1988); and with other mineral species (Pritchard *et al.*, 1984); phosphate distribution within tissues (Caradus, 1986; Hart, 1982; Hart *et al.*, 1981a, b); the distribution of phosphate between different metabolic forms (Caradus and Snaydon, 1987; Hart and Jessop, 1982; 1983; 1984a b) and the relationship between phosphate metabolism, and photosynthesis in white clover (Hart and Greer, 1988; Hart and Jessop, 1984b). Therefore we understand many of the important plant processes which involve phosphate. However much of this work has necessarily been conducted using plants grown under conditions quite different from those prevailing in the field. Consequently these studies do not indicate the relative quantitative contribution of each aspect to performance in the field when phosphate is limiting.

The difficulties in predicting the quantitative effect of altering a particular physiological parameter convinced us that the best way to proceed was to first obtain material with genetic differences in its response to phosphate. This would permit us to concurrently follow several lines of work to culminate in a phosphate efficient cultivar

of white clover. The first priority would be to determine heritability for the differences in response to phosphate. If heritability is low breeding for response would not be possible. The second line of work could examine the performance of promising material in agricultural situations. Thirdly, material with differences in response could be characterised physiologically and morphologically in an attempt to ascertain the properties which confer the desired performance.

This approach assumes that phosphate efficiency is a character or group of characteristics that can be transferred between genotypes using conventional plant breeding procedures. We have no information to test this assumption as yet.

The decision to conduct the initial trials and selection using pot trials was considered exhaustively. The differences between material grown in pots and in the field are large and there remains concern that relevance between the results for the two environments may be minimal. However the alternative problem of finding a field site that would provide a uniform and controllable environment was considered to be even more intractable. It was decided that pot trials be used to identify material with demonstrable differences in their performance under scarce phosphate and then at the earliest opportunity to test these differences in the field.

An outline of our strategy is given in Figure 1.

Stage one — differences among cultivars in their response to phosphate

The initial step taken was to obtain phosphate response curves for 120 cultivars and lines of white clover. This project has been described at this symposium in the paper of Mackay *et al.* (1988). The seed lines came from a wide range of sources. A trial involving 2400 pots was used to determine responses at 11 levels of phosphate. This required 100 square metres of glasshouse space. Watering and rerandomisation of the pots required about 60 man hours labour each week. Both the frequency and extent of differences in responses to phosphate measured were considerably larger than we expected.

Stage two — genotypic differences in response to phosphate

Before obtaining the results of stage one, the second stage of the programme was to be a large pot trial to screen 40,000 seedlings from the ten most promising cultivars. After the first experiment the programme was revised. It was not possible to dismiss the possibility that some of the variability obtained in stage one was a result of factors other than phosphate nutrition. However if the variation in phosphate nutrition was as large as it appeared to be, the large pot trial which would strain our resources would be unnecessary. Accordingly it was decided to conduct a trial which would test whether the differences previously obtained could be found under different experimental conditions. This trial may also provide material with differences in performance.

This stage is examining the phosphate responses of genotypes (10 per cultivar) selected at random from 11 cultivars which in turn were selected for divergent responses to phosphate. An additional ten genotypes were selected from a single cultivar (Grasslands Huia) on the basis of the weight of the seedling grown at a single low phosphorus level. The genotypes have been cloned and planted at nine phosphate levels. This trial is presently in progress.

Stage three — heritability

If stage two confirms the general trend of differences found in the preceding stage, the programme can then develop along three lines. The priority will be to determine the heritability of the differences obtained. If heritability is low the differences will have little value for plant breeding. Heritability will be assessed by measuring the phosphorus responses of progeny from both a diallel cross and a polycross. There will be 100 seed lines plus 10 parents to be evaluated. This will be done using stolon tip cuttings similar to those used in the preceding measurement of phosphorus response curves. The material will come from 60 seedlings for each seed line which should give samples that are representative. The trial will involve a total of 2,200 pots.

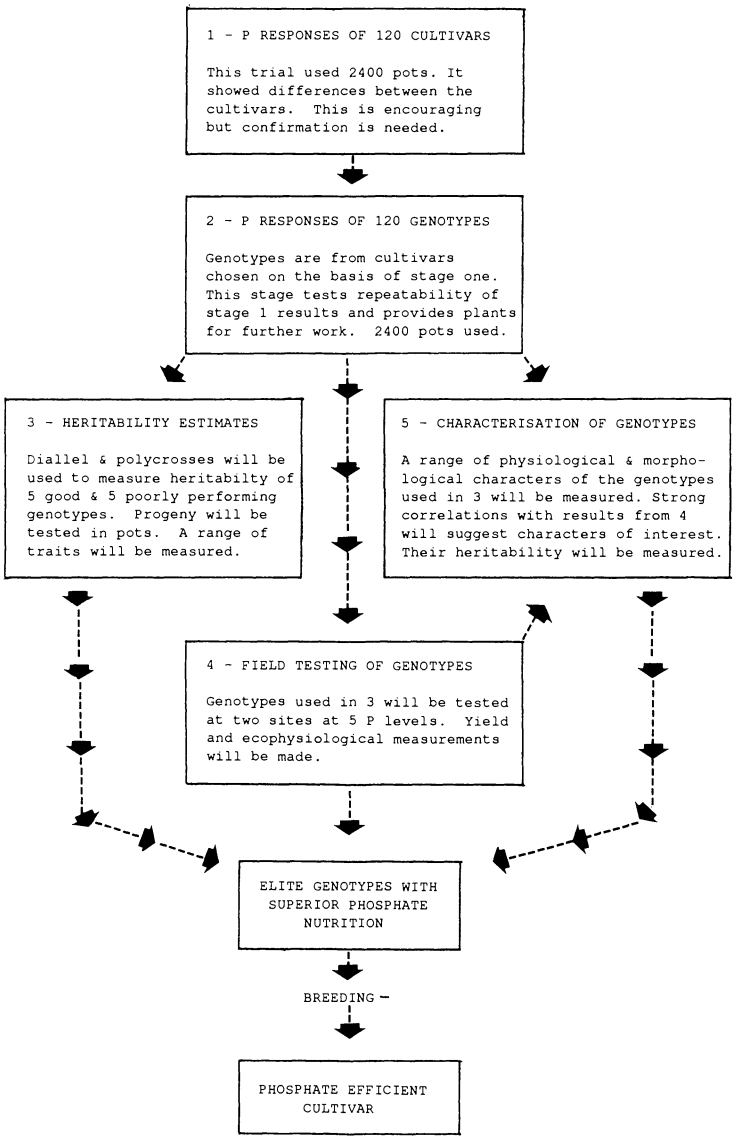
1 - P RESPONSES OF 120 CULTIVARS
This trial used 2400 pots. It showed differences between the cultivars. This is encouraging but confirmation is needed.
2 - P RESPONSES OF 120 GENOTYPES
Genotypes are from cultivars chosen on the basis of stage one. This stage tests repeatability of stage 1 results and provides plants for further work. 2400 pots used.
3 - HERITABILITY ESTIMATES
Diallel & polycrosses will be used to measure heritabilty of 5 good & 5 poorly performing genotypes. Progeny will be tested in pots. A range of traits will be measured.
5 - CHARACTERISATION OF GENOTYPES
A range of physiological & morphological characters of the genotypes used in 3 will be measured. Strong correlations with results from 4 will suggest characters of interest. Their heritability will be measured.
4 - FIELD TESTING OF GENOTYPES
Genotypes used in 3 will be tested at two sites at 5 P levels. Yield and ecophysiological measurements will be made.
ELITE GENOTYPES WITH SUPERIOR PHOSPHATE NUTRITION
BREEDING
PHOSPHATE EFFICIENT CULTIVAR

Fig. 1. Outline of a programme to breed a cultivar of white clover with improved phosphate nutrition. *Arrows* indicate the interrelationships between various stages of the programme.

Stage four — field assessment of genotypes

A second concurrent trial will be run to provide information on the field performance of material selected from stage two. It is probable that some of the material which performed well in pots will display only mediocre performance in the field. This may be due to factors that have little relationship to phosphate nutrition. Alternatively it may indicate that the characters required for good performance in pots when phosphate is limiting do not equip plants to cope with phosphate deficiency in the field. We accept that this may be a significant problem the solution of which will be essential to the success of our programme. Discrepancies between performance in the field and in pots which are not due to phosphate nutrition might be resolved by breeding to introduce improved

phosphate nutrition into plants known to perform well in the field. If a discrepancy arises more directly out of aspects of phosphate nutrition the results will probably shed light on the requirements of plants growing the field. Information from the characterisation of phosphate nutrition should also help to understand the difficulty. This would help to redefine selection criteria.

Stage five — physiological and morphological characterisation

In a further concurrent study the phosphate nutrition of contrasting genotypes will be characterised in an attempt to determine properties which are correlated with good performance at limiting levels of phosphate. In particular characters such as rate of phosphate absorption, proton extrusion, biochemical partioning, distribution of phosphate within the plant, root morphology, leaf size and branching patterns of the stolons, and nitrogen fixation will be quantified. Strong correlations between any of these characters and plant performance at limiting phosphate will suggest that they may contribute to good plant performance and might be useful as parameters which can be used as selection criteria. Experiments to confirm this would be warranted. Determining these characters in the progeny of the polycross and the diallel cross (stage three) will give information on their heritability and it will provide a further test of their significance to performance.

The value of any particular character in screening will depend on its heritability, the extent of its variability in the genetic material available, and the speed and accuracy with which it can be determined. It is possile that although it may not be practical to screen large numbers of plants for a given character, that character may be valuable as an additional criterion to assess material selected by other means.

Concluding comments

When we have completed stages three, four and five we should have genotypes with superior characteristics in their phosphate nutrition and data on heritability, performance in the field and the physiological and morphological basis for their better performance. This will allow us to proceed with the selection and breeding of a cultivar.

We do not consider the programme as it is presented here to be inflexible. Already it has been changed in the light of the results obtained in stage one. As each stage is completed the progress made and future work will be reviewed. This is necessary not only because we are accountable for the resources being used but also because this is research, that is venturing into the unknown, and it is unwise to be dogmatic about the unknown.

Acknowledgement

We than M J Baker for his valued assistance as secretary to our group.

Note added in proof

In stage two, now completed, the trends were similar to those obtained in stage one. Stage three is now well advanced.

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PLSO PN 198
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Response to phosphorus of a world collection of white clover cultivars

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Key words: cultivars, multivariate analysis, phosphorus response, *Trifolium repens*, white clover

Abstract

White clover (*Trifolium repens* L.) is the predominant legume of temperate pastures. Nitrogen (N) fixed by white clover is essential for maintaining the production of grasses in agricultural systems where N fertilisers are not used. However, white clover is a poor competitor for soil phosphate (P). This is of particular significance when many soils in New Zealand and throughout the world are P deficient. The increasing cost of manufacture and application of P fertilisers has stimulated a breeding programme to develop a white clover with improved P nutrition.

This paper reports the first study in this programme which investigated the extent of genetic variation in the response of white clover cultivars to added P. The response of 119 white clover cultivars and lines from 25 countries, which includes all morphological types, to added P was measured in soils in large pots in a glasshouse. Phosphate was added at 11 rates ranging from 0 to 750 mg kg^{-1} of soil.

There were significant differences between cultivars for shoot dry weight response to added P. Cultivars were grouped into eight clusters on the basis of their fitted quadratic P response curves. They varied from high to low response at low P levels, and from a rapid decrease in response to P to an increase in response to P with increasing P level. Cultivars with a high initial response to added P all originated from countries with cold winters. There was no other apparent relationship between origin and P response characteristics.

This study has demonstrated that a large range of variation for P response exists within white clover. This forms the basis for a selection and breeding programme.

Introduction

White clover (*Trifolium repens* L.) is the most important temperate legume grown in pastures, because of its ability to fix nitrogen (N) while being able to withstand the stresses of grazing (Brougham *et al.*, 1978). However, when grown with grass, white clover competes ineffectively for soil phosphate (P), especially when plant available soil P levels are low (Jackman and Mouat, 1972a, b) as is the case in most New Zealand soils. Improved legume growth leads to increased N fixation and ultimately increased grass production (Hoglund and Brock, 1987). However, with the increasing costs of manufacture and application of P fertilisers there is a pressing need to breed a white clover capable of responding to lower levels of applied P than cultivars already available commercially. The present study was the first stage in this programme (Dunlop *et al.*, 1988) and was aimed at determining the extent of variation in response to P of a world collection of white clover cultivars.

Materials and method

Plant material

Seeds of 112 white clover cultivars and 7 breeding lines, from 25 countries were germinated at

25°C in petri-dishes, containing moist filter paper, transplanted after 48 h to sand trays and grown for 10 days before planting into pots. A full description of each cultivar is given by Caradus (1986).

Soil

The soil used in this study was a Wainui silt loam (Typic Dystrochrept), with a pH of 5.0 and Olsen P of 6 mg kg^{-1}. It was collected from the field to a depth of 100 mm and passed moist through a 4-mm sieve.

Conduct of study

A pilot study determined the range of P levels necessary for constructing a response curve. Rates of 0, 20, 40, 80, 120, 200, 300, 400, 500, 600 and 750 mg P kg^{-1} were used, the 0, 40, 120 and 200 rates being replicated 3 times, the 400, 500 and 600 rates twice, and the others only once. Also added was sulphur as gypsum ($CaSO_4$) and potassium as potash (KCl). Soil was incubated at a gravimetric moisture content of 48% for 30 d, before packing into 120 mm diameter pots to a bulk density of 0.8 Mg m^{-3}. Three seedlings were transplanted into each pot and pots were arranged in a randomised block design in a glasshouse. A *Rhizobium trifolii* inoculum cocktail containing strains NZP540, 541, 542, 547, 548 and 560 was added to each pot one week after planting. Three times a week pots were watered to weight, so that soil was at a gravimetric moisture content of 55%. At each watering pots were re-randomised within blocks, and blocks were moved within the glasshouse.

The study was conducted during April and May (autumn) 1987 and temperatures in the glasshouse ranged from 15°C to 30°C.

Measurements

A destructive harvest was taken 56 d after planting. Leaf number and dry weight, stolon length and dry weight were measured. The above measurements allowed calculations to be made of total shoot (leaf plus stolon) weight, proportion of leaf to total shoot weight, and individual leaf weight. All measurements were calculated on a per pot basis.

Statistical analysis

All data were subjected to an analysis of covariance with P level as the covariate. Where appropriate data was log-transformed to ensure homogeneity of variance.

Principal component analysis, a multivariate technique which looks for a few linear combinations that can be used to summarise the data losing as little information as possible in the process (Mardia *et al.*, 1979), was carried out on log-transformed data (except where data were in the form of a proportion). Quadratic response curves up to 500 mg P kg^{-1} ($y = a + bx + cx^2$) were fitted to the first principal component. Above this level most response curves levelled out, and many showed possible P toxicity symptoms (*i.e.* reduced yield) at the highest P level. Rates of response for each cultivar were calculated up to 500 mg kg^{-1} added P and cultivars were clustered into eight groups based on the response rates using the flexible-beta method (Lance and Williams, 1967).

Results and discussion

Analysis of variance

While overall cultivar effects were generally non-significant there were highly significant cultivar × P level interactions for all characters except proportion of leaf to total shoot weight (Table 1), indicate that a large amount of intraspecific variation for P response exists within white clover.

Principal component analysis

The first and second principal components accounted for 85% and 9% of the variation, respectively. The first principal component was a measure of plant size; eigenvectors associated with dry weight measurements, leaf number, and stolon length had the largest magnitudes (Table 2). The

Table 1. Analysis of variance for measured characters and principal components

Source of variation	df	Log-shoot weight	Log-leaf weight	Log-stolon weight	Log-leaf number	Log-stolon length	Log-individual leaf weight	Propn leaf	Principal component	
									1	2
Cultivar	120	ns	ns	ns	***	***	ns	*	ns	ns
P (linear)	1	***	***	***	***	***	***	***	***	***
P (quadratic)	1	***	***	***	***	***	***	***	***	***
Cultivar × P	120	***	***	***	***	***	***	ns	***	***

ns not significant
* significant at 5% level
*** significant at 0.1% level

second component was dominated by proportion of leaf, with individual leaf weight also contributing; both characters describing plant morphology. There was a significant cultivar × P level interaction for both the first and second principal components (Table 1).

Fitted curves

Quadratic response curves fitted to the first principal component gave R^2 values ranging from 89% to 98%. The lack of fit, using the quadratic function, was not significant for 85% of the cultivars.

Cluster analysis

Eight groups of cultivars (Table 3) based on the slopes of the fitted quadratic curves at seven P levels were derived from the flexible-beta cluster analysis. For clusters A to G, response to P at very low P levels ranged from very high to very low, and change in response to P with increasing level of added P ranged from a rapid reduction (cluster A),

Table 2. Eigenvectors of the seven log transformed variables for the first and second principal components

Variable	Principal component	
	1	2
Log-shoot dry weight	0.40	0.20
Log-leaf dry weight	0.40	0.24
Log-stolon dry weight	0.39	0.09
Log-leaf number	0.38	−0.18
Log-stolon length	0.40	−0.17
Log-individual leaf weight	0.35	+0.47
Proportion leaf	−0.31	+0.78

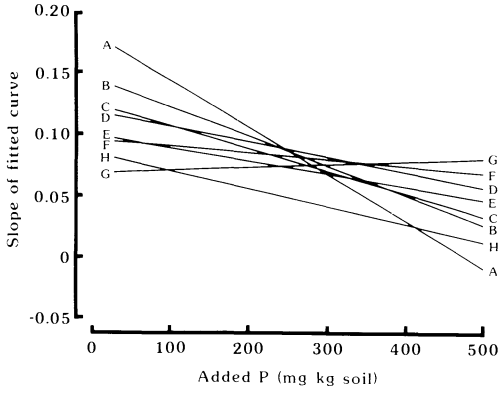

Fig. 1. Change in slope with increasing P level of fitted quadratic P response curves for the first principal component, of clusters A to H.

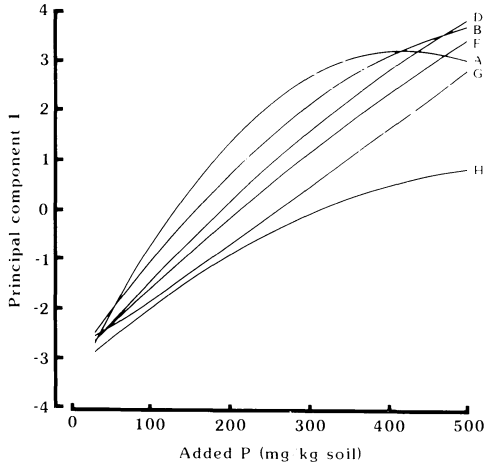

Fig. 2. Fitted response curves for example cultivars from cluster A (Gwenda, $y = -3.68 + 0.20\chi - 0.0015\chi^2$), B (Gomelskij, $y = -3.24 + 0.15\chi - 0.0008\chi^2$), D (Podkowa, $y = -3.30 + 0.12\chi - 0.0004\chi^2$), F (Merwi, $y = -3.11 + 0.10\chi - 0.0002\chi^2$), G (Duron, $y = -2.92 + 0.07\chi - 0.00005\chi^2$) and, H (Luclair, $y = -3.28 + 0.09\chi - 0.0005\chi^2$). *Curves* of example cultivars from clusters C and E were similar to that for cluster F.

Table 3. Lists of cultivars and lines grouped into clusters on the basis of change in response with increasing levels of added P. Numbers indicate country of origin

Cluster							
A	B	C	D	E	F	G	H
Beta 21[a]	Daeno 8	Barbian 16	Alban 8	Anirak 22	Alice 22	Crau 10	Luclair 10
Gwenda 22	Elen 22	Belogorskij 25	Armada 16	Anna 22	Aran 12	Duron 5	
Trifo 8	Gandalf 8	Chiemgauer 11	Bage 4	De Brasov 19	Atoliaj 25	El Lucero Inta 1	
	Gomelskij 25	Clarence Valley 2	Blanca 3	G.26[b] 17	Bayucua 23	G.23[b] 17	
	Ladino Giganteum Lodigiano 14	Common Idaho 24	Bonners ferry common 24	Karina 11	Bitunai 25	Morso 8	
	Makibashiro 15	Cultura 16	Feathermark 17	Kent 22	California 24	Priekulskij 25	
	Milka 8	Donna 22	Gigant 14	ML-48-65 5	Dusi 20	Pronitro 16	
	Milkanova 8	Ensign 22	Isolation V[b] 17	Ovcak 7	Kitaooha 15	Svolder 21	
	Nesta 22	Fries Groninger 16	Katrina 22	Pitau 17	Kopu 17		
	Prikarpatsk 1 25	Haifa 2	Kersey 22	S.100 22	Irrigation 2		
	Radi 18	Huifa 17	Louisiana 24	Tahora 17	Lena 21		
	Retor 16	Kivi 21	Louisiana S.1 24	Tammisto 9	Linda 22		
	Viglasska 7	Major 10	Magurele-1 19	Whatawhata[b] 17	Lirepa 11		
	Volat 25	Medunaj 25	Merit 24		Lune de Mai 10		
		Menna 22	Olwen 22		Merwi 3		
		NFG-Gigant 11	Osceola 24		Nora 21		
		Sabeda 22	Pertina 16		Otofte 8		
		Smalbladet 8	Podkowa 18		Pastevec 7		
		Spreading seln[b] 17	Regal 24		Polyploid ladino[b] 24		
		Steinacher 11	Ross 12		Quin Zhen[b] 6		
		Tamar 13	Sandra 21		Rivandel 8		
		Tribla 3	Tillman 24		Robusta 21		
			Titan 24		S.184 22		
			Zapican 23		Sacramento 24		
					SC-1 24		
					Simone 14		
					Siral 2		
					Siwan 22		
					Smena 25		
					Sonja 21		
					Star 24		
					Trevise 10		
					Undrom 21		
					Zero 8		

[a] Country of origin; 1 Argentina; 2 Australia; 3 Belgium; 4 Brazil; 5 Canada; 6 China; 7 Czechoslovakia; 8 Denmark; 9 Finland; 10 France; 11 Germany; 12 Ireland; 13 Israel; 14 Italy; 15 Japan; 16 Netherlands; 17 New Zealand; 18 Poland; 19 Rumania; 20 South Africa; 21 Sweden; 22 United Kingdom; 23 Uruguay; 24 USA; 25 USSR.

[b] Breeding lines, not cultivars.

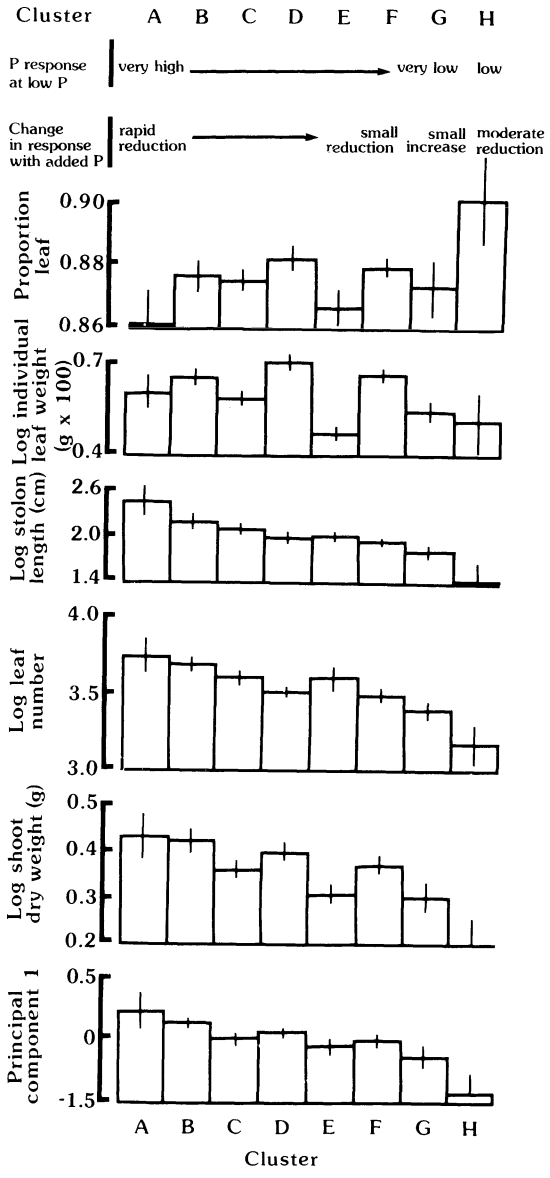

Fig. 3. Description of clusters A to H. Values are means of cultivars, within each cluster, over the first nine P levels. *Bars* indicate standard errors.

through only a slight reduction (cluster F) to a slight increase (cluster G) (Fig. 1). Cluster H was unusual in that it had both a low response to P at very low P levels and a moderate reduction in change in response as P level increased. Examples of cultivars whose fitted response curves were close to the mean for clusters A, B, D, F, G and H are given in Fig. 2.

There was a gradual decrease in shoot size variables log shoot dry weight, log leaf number and log stolon length meaned over all cultivars and P levels (up to 500 mg kg^{-1}) across clusters A to H (Fig. 3). There was no obvious relationship between cluster ranking based on P response characteristics and either mean individual leaf weight or mean proportion of leaf (Fig. 3).

Clustering of cultivars on the basis of P response characteristics was generally unrelated to country of origin. However, cultivars with a high response to P at low P levels and a rapid decrease in response with increasing P level (clusters A and B) were all from countries or regions with cold winters (Northern Europe, Scandinavia, Japan and the Po Valley in Italy) (Table 3). The significance of this relationship at this stage is not apparent.

Conclusion

A large amount of intraspecific variation for P response is available within white clover for an ongoing breeding programme. Tests of reproducibility and heritability will determine the long-term usefulness of differences in P response observed in the present study.

Acknowledgement

To Craig Anderson, Ian Black, Philip Budding, Roger Claydon, Elli Cornege, Des Costall, Miranda Filemoni, Margaret Knighton, Allison MacKay, Venessa Pokaia, Metiria Turei, Brett Watson for technical assistance, and to Cherry Liddane for supplying Rhizobium inoculum.

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